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PROBLEMS OF SPACE BIOLOGY

VOLUME 4

N. M. Sisakyan, Editor-in-Chief

USSR Academy of Sciences Publishing House,
Moscow, 1965

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N. M. Sisakyan, Editor-in-chief

Translation of "Problemy kosmicheskoy biologii, Tom IV."
Izd-vo Akademii Nauk SSSR, Moscow, 1965.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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ANNOTATION

Volume 4 of the collection "Problemy kosmicheskoy biologii" (Problems of Space Biology) now presented to the reader is a series of articles devoted to timely problems in space biology and medicine. It includes articles of theoretical and summary nature, experimental investigations carried out on the ground and works relating to the training of cosmonauts, evaluation of the radiation danger associated with spaceflight, isolation and hypodynamia, current status of the problem of ecology in a closed system with the recirculation of substances, cybernetics with reference to the needs of space biology, etc. A separate section describes experiments performed during the orbital flights of the cosmonauts on the Vostoks.

The third and major part of the book is concerned with the results of laboratory experiments on animals and testing apparatus.

The fourth and last section contains articles on general aspects of space biology and medicine and on methods.

The Editors hope that the collection will be of interest not only to space researchers, but also to specialists in other branches of medicine and biology.

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PART I. MEDICAL AND BIOLOGICAL INVESTIGATIONS UNDER TERRESTRIAL CONDITIONS

TRAINING DEVICES FOR PREPARING COSMONAUTS FOR OCCUPATIONAL ACTIVITY IN CONTROLLING SPACECRAFT AND THEIR SYSTEMS

N. N. Gurovskiy, V. G. Denisov, A. P. Kuz'minov
and M. M. Sil'vestrov

The successful accomplishment of tasks with which the cosmonaut is /3* faced while in flight requires careful training of the spacecraft crew on Earth.

Whereas the basic form of training pilots is by practice flights on aircraft (training in special trainers being of secondary importance), the training of cosmonauts in simulators is of paramount importance in preparing crews for spaceflights.

This is understandable, since up to now practice spacecraft intended for instructing and training cosmonauts while in flight have not been developed. Consequently, the demands placed on terrestrial testing units and simulators for cosmonauts are necessarily very high. These testing units must simulate the conditions and factors of spaceflight and emergency situations, the operation of individual systems and the dynamics of flight, as well as provide the cosmonauts with the necessary practical experience.

The necessary practical experience must be acquired by the use of a system of simulators. These simulators are quite varied as regards their purpose and significance in the system for training the cosmonauts and in their structural design. In this connection it is desirable to describe the general fundamentals of the system of simulators necessary for training spacecraft crews.

In order to establish a basis for the system of space simulators, we must study the activities of cosmonauts in various spacecraft to determine those /4 tasks which lend themselves to analysis in simulators, and to conduct an analysis of the training program, as well as of the simulators from the point of view of their effectiveness.

Depending on the type and character of the cosmonaut training, all simulating devices can be divided into two basic groups: simulators and devices for training the cosmonaut's organism for the conditions of spaceflight and simulators for assisting the cosmonauts in developing professional skill in handling the controls of the spacecraft and its systems.

*Numbers given in the margin indicate the pagination in the original foreign text.

In addition, there may also be combination-type simulators, in which the training of the cosmonauts for skilled activities aboard the spacecraft is combined with the training of the cosmonauts' organism for the extreme conditions of spaceflight.

The first group includes simulators and devices for physical and special training and devices which simulate the surrounding medium and conditions of spaceflight.

We shall concern ourselves in detail only with those simulators which are used for perfecting the cosmonauts' skill in handling the controls of the vehicle and its systems.

Depending on the effect of motion factors on the human organism, these simulators may be dynamic or static. Dynamic simulators are devices in which the cabin or the working space of the trainee moves in conformity with the simulated dynamics of the vehicle. Thus, in dynamic simulators the human organism is affected by such factors as angular velocity, angular acceleration and linear acceleration. In static simulators the cabin is stationary.

Depending on the volume of the simulated systems, the stages of flight and the simulated tasks, the simulators for perfecting the professional skills of the cosmonauts may be universal, complex, specialized or functional.

The universal simulator is a single multipurpose simulator, intended for training the crews of space vehicles of various types. It is an extremely complex device, whose characteristics must be changed and adjusted in accordance with the characteristics of various projected or existing space vehicles. The most important elements of such a universal simulator are a movable or stationary model of the cabin of the space vehicle, a computer, an instructor's console, a model of the heavens and Earth's surface, a programmer and recording equipment.

The universal simulator may include a cabin or chamber for creating the appropriate temperature and pressure conditions, rocket noise, vibrations, gas medium, humidity and air circulation and for simulating certain flight conditions which would be encountered on a specific type of space vehicle.

A complex simulator permits training of all members of the crew for /5
activity on a given type of space vehicle during all stages of flight.

The simulator which was used for training space pilots for flights on space vehicles of the "Vostok" type can be used as an example of the complex simulator. In this simulator the trainees developed skill in controlling the flight conditions and the operation of the spacecraft systems and equipment, in manual orientation of the space vehicle, in carrying out communications with Earth, in controlling the spacecraft systems, in manual descent from orbit and in handling the systems and units in emergency situations.

The training device permits simulation and modeling of the handling of the automatic and manual orientation systems, the onboard program device, the braking-rocket control system, the noise of the carrier-rocket engines and of

the braking-rocket engine and the alternation of day and night during orbital flight. The communication system of the simulator fully simulates the operation of a two-way communication system between Earth and the space vehicle over shortwave and ultrashortwave channels. The operation of the equipment controlling the functioning of the life-support and thermoregulatory systems is also simulated, as well as the operation of the cabin instrumentation and master radio line.

The equipment in the cabin mockup does not differ from regulation equipment. The control dynamics are simulated on a computer and special units. Simulation of the visible image of Earth and sky in a "Vzor" (Look) optical orientation device and of the shifting of the image during steering is accomplished by means of an optical-mechanical device. The training is supervised via the instructor's console.

Thus, this simulator develops the cosmonaut's skill in handling the controls both during normal flight and in emergency situations, from liftoff to descent.

According to the latest ideas on the possible types of spacecraft and the necessity for training the cosmonauts adequately, it will be necessary to provide the following types of complex simulators: those for the crews of interplanetary vehicles, those for the crews of satellite spacecraft and those for the crews of rocket planes (orbital aircraft).

The specialized simulator is intended for developing skills in a whole complex of specific work operations essential for the execution of special tasks of future spaceflight.

In specialized simulators, the crew members must develop skill in the use of certain systems designed for the solution of one or more problems of spaceflight. For example, the following types of specialized simulators are possible: simulators for orientation of the vehicle, for navigation, for control of the vehicle in passing from orbit to orbit, for rendezvous with /6
space stations and for "mooring" to the latter, for assembly and repair of space stations or of space vehicles in orbital flight, for directing the takeoff of interplanetary spaceships from space stations, for observing objects outside the vehicle, for conducting scientific investigations while in flight, for landing the vehicle on Earth or other planets, for controlling the operation of the life-support system, etc. From the whole multiplicity of tasks to be accomplished in the simulator, only one or a few are selected, and only those systems and sources of information are modeled which will be used by the cosmonaut during execution of these tasks.

Specialized simulators would include simulator stands of the kind used for training cosmonauts for space walks from vehicles of the "Voskhod-2" type. This simulator was a mockup of the cabin of a space vehicle with an air lock situated in the heat chamber. In designing this simulator, account was taken of the requirement for maximum approximation of the training conditions to actual flight conditions.

The simulator stand trains the future crew members in cooperation during extravehicular activity on the part of a single cosmonaut.

The stand made it possible for cosmonaut A. A. Leonov to develop the special abilities, to the point of automatic performance, needed for making a space walk, while at the same time carrying out an experiment in space and returning to the vehicle, as well as to adapt to the conditions experienced while remaining in a high vacuum. The stand also enabled P. I. Belyayev to perfect his skill in operating the air lock and exit equipment, in controlling the condition of a cosmonaut outside the vehicle and in carrying out communications and directing the return of the cosmonaut.

Another example of the specialized simulator is the flying laboratory (aircraft), provided with air lock devices. A simulator of this type trains the cosmonaut to develop skill in stepping from the vehicle into space under conditions of weightlessness.

As a result of careful training, man's first attempt at walking in space was carried out effectively and in practice indicated the theoretical possibility of carrying out operations outside the space vehicle.

In connection with the fact that without appropriate reinforcement certain skills may fade, it is necessary to utilize not only the simulators indicated above, but also certain onboard specialized simulators, designed for sustaining the skills required by the cosmonauts in performing vital operations during prolonged spaceflight. Such simulators would include, for example, devices for simulating landing on Earth or a planet, "mooring" the vehicle to a space station, etc. The onboard simulator must provide for the utilization of a system of indication, signaling, manual controls and an onboard computer. /7

The functional simulator is designed for developing skill in carrying out work operations involving one type of activity or for training individual human functions, such as tracking, attention, etc. It is not necessary for the functional simulator to model any concrete system with which the operator works. It need only simulate that which is necessary for increasing the functional capacity of a man for the required type of activity.

As pointed out, in principle it is possible to develop a combination-type simulator in which the preparation of cosmonauts for professional activity in controlling the space vehicle and its systems takes place in combination with the training of the cosmonaut's organism to adjust to the extreme conditions of spaceflight.

The design of such a combination-type simulator necessitates the incorporation of computers for modeling the flight dynamics of the vehicle, all simulators of its systems and equipment and the simulators of the ambient medium and the conditions of spaceflight.

This problem is difficult to solve and requires the expenditure of considerable sums of money. A description of such a device will give some idea of its complexity. On a centrifuge, having an arm measuring 15-20 m, a cabin mockup is installed with six degrees of freedom, permitting the simulation of

G-loads and three-dimensional motion of the vehicle. The cabin mockup must be equipped with all active systems of life-support and thermoregulation, means of communication and all equipment which would be present on a vehicle of this type.

The device must provide for simulation of weightlessness over an extended period of time (let us assume this is done by filling the cabin with a liquid having the same specific gravity as that of the human body). The cabin, together with the simulators of G-loads and weightlessness, is placed in a special thermal environment simulation chamber, which simulates the medium surrounding the space vehicle. With the aid of an orrery an external viewing arrangement is produced on the interior surface of the spherical dome of the thermal environment simulation chamber. The image of Earth and sky shifts in accordance with the laws of celestial mechanics and with the dynamics of the steering of the space vehicle. The dome must be black in color, with a low reflection coefficient (for simulating the conditions of high contrast which obtain in outer space).

Inside the chamber there must be equipment for simulating meteor danger, as well as a device simulating the effect of high temperatures generated on entering the dense layers of the atmosphere. Noises and vibrations must be simulated inside the cabin of the simulator.

The course of the program, the dynamics of movement, modeling of the /8
control systems, changes in the readings of the instruments, introduction of emergency situations, changes in the environmental conditions, disturbances and noises are all determined by means of a computer, located in another place. The training is directed and controlled from the instructor's console. There is an intricate complex of observations and registrations of the psychophysical condition of the trainee and of the control of the simulator-operation parameters. In designing a combination-type simulator with full simulation of the environmental conditions, it is apparently advisable to make the simulator universal, suitable for training in connection with any type of space vehicle.

At the present time there is no single opinion concerning what types of simulators should be built. According to some extreme views, only complex simulators should be built, or the choice should be limited to the universal type (ref. 1), while the basic preference is given by some investigators to functional and specialized simulators.

Proponents of the first extreme view raise the question as to whether a simulator should be built capable of simulating not only any given type of space vehicle, but actually several types, arguing that space vehicles have many common characteristics and that the differences between them are not substantial.

We cannot agree with these extreme views. Training the operator in functional simulators assures rapid, reliable development of individual skills, because his attention is not distracted from the assigned activity during training.

Apart from that, functional simulators are simple and inexpensive, and they have a large handling capacity. Consequently, functional simulators are

advisable for developing those skills requiring a maximum number of hours in training, as well as skills in which it is especially difficult to achieve perfection.

However, an operator on a space vehicle, engaged in carrying out a specific type of activity for which he has been trained in a functional simulator, must in addition keep track of information supplied by other equipment, particularly that on board, and take part in the operation of other systems not directly connected with the performance of a given type of activity. The operator who is not prepared for this cannot carry out the assigned tasks or even those operations for which he was trained in a functional simulator.

For this reason, the final development of skill in performing specific operations must take place in specialized and complex simulators, in which the actual conditions of the operator's activity during spaceflight are simulated to a greater extent.

At the same time, specialized simulators provide the operator with /9 the ability to perform a specific group of tasks which are necessary in flight.

Complex simulators give the operator a chance to develop a keener awareness of the volume of operations on a space vehicle and a better knowledge of the working operations from liftoff to landing. In addition, they make it possible for him to study possible emergency situations, failure of individual units and systems, etc.

The building of universal simulators is hardly feasible at the present time. Even a brief examination of the tasks carried out on various types of space vehicles, and of the characteristics of the equipment which must be controlled, indicates the complexity of the task.

Reproducing the characteristics of various objects which must be controlled, which vary within wide limits, and modeling the operations of all spacecraft systems, makes the universal simulator unwieldy, expensive and relatively ineffective. Moreover, with further strides in the mastery of space the number of types of space vehicles will grow, and the handling capacity of the universal simulator will be inadequate for the needs of the crews of the various types of vehicles.

Our analysis leads to the conclusion that simulators for developing the professional skills of spacecraft crew members must be built in conformity with the purpose of each vehicle and the particular type of activity required of the cosmonaut on the given type of vehicle.

In order to provide the cosmonauts with training of maximum benefit, as well as to be in a position to conduct research in rational methods of indicating and signaling, manual and semiautomatic control systems and arrangement of spacecraft equipment, it is necessary to have three types of simulators: complex, specialized and functional. Such a system of simulators assures effective training for professional activity under the conditions of spaceflight.

Extended spaceflights, as previously mentioned, require the presence of an onboard simulator for training the spacecraft crew to perform vital operations during prolonged flight.

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BASIC PRINCIPLES OF SPECIAL COSMONAUT TRAINING

N. N. Gurovskiy, M. D. Yemel'yanov and Ye. A. Karpov

Man encounters a complex set of environmental factors during space- /10
flight. Spaceflight makes great spiritual and physical demands on the cosmonaut. Each member of the crew must remain physically fit and capable of orienting himself in complex flight situations and, if necessary, help to control the space vehicle and its onboard systems.

The success of manned flights depends not only on creating optimal conditions in the spacecraft capsule but on the thorough training of the cosmonauts. It is understandable that training methods are constantly being improved.

The individual characteristics of healthy persons are not stable. Under the influence of external and internal stimuli significant deviations from generally accepted physiological norms constantly arise. These deviations generally do not reflect a pathological condition.

It is a well-known fact that man possesses a fairly wide capacity for developing resistance to external influences. This is one of the main fields of space medicine.

The special cosmonaut training program is based on analysis of spaceflight factors which most affect the cosmonaut, with consideration of his actual performance during flight. These factors have been tentatively divided into several groups. One involves an understanding of space as a peculiar environ- /11
ment in which man cannot exist without protection (complete vacuum, ionizing radiation, low temperature, etc.). The space vehicle provides fairly reliable protection against the injurious effect of these factors so that no special training measures are needed on Earth.

Another group of factors pertains to the dynamics of spaceflight. These include noise, vibration, acceleration, weightlessness, and prolonged vestibular stimulation by the rotary and rolling movements of the vehicle. The training program must embrace measures aimed at increasing resistance to this group of factors.

A third group of factors arises in connection with the comparatively long stay of the cosmonaut in the capsule of a spacecraft. These include the microclimate, heat regime, confinement in a small space with limited mobility possible, unusual food, compulsory work routine, special clothing, nervous and

mental stress due to the novelty and unexpectedness of the situation, space-time relations different from those on Earth, etc.

Mention must also be made of the landing procedures, especially if parachutes are involved. It is a particularly serious matter when unfavorable weather conditions prevail in the landing area. Therefore, the cosmonaut has to acquire the skills of a parachutist and be able to orient himself and function efficiently after he lands.

The various spaceflight factors are simulated under laboratory conditions or during aircraft flights so that individuals can be exposed to them as a means of choosing the best candidates. Unfortunately, some factors cannot be reproduced on the Earth (prolonged weightlessness, peculiar psychological background of flight, etc.). Therefore, besides training exercises in which the active factors are similar to those encountered by the cosmonaut in /12
flight (acceleration, high temperature, etc.), the program should include some nonspecific methods aimed at increasing specific and general resistance to flight factors (general endurance, ability to control the body during a free fall in space, increased resistance of the vestibular apparatus to adequate stimulation, etc.).

An important element in cosmonaut training is the use of special psychological methods of determining the resistance level and individual nervous and mental peculiarities of each candidate. The information thus obtained is of considerable value in predicting the candidate's behavior in flight.

The training program is also concerned with the acquisition and strengthening of skills required to operate systems and units on the spacecraft.

Thus, the set of exercises and tests should include flying on aircraft equipped to create weightless conditions, investigation of the nervous and psychological aspects of prolonged confinement in a soundproof chamber, practice in a mockup of a spacecraft cabin and trainer, tests and exercises in a heat chamber, tests and exercises on a centrifuge, and special physical and vestibular training.

Investigations during flights on aircraft along Kepler's trajectory both in the USSR and United States showed that all individuals can be divided into three groups by the nature of their reactions to weightlessness (more precisely, to the alternating action of acceleration and weightlessness). The most resistant do not develop noticeable disturbances. Others have autonomic reactions (general weakness, facial pallor, hidrosis, nausea, unusual illusions). This condition rules out the possibility of future participation in such flights. The third group has vague symptoms of motion sickness during the first flights, but then adapts to weightlessness and after 3-5 repetitions no longer experiences any disagreeable sensations. Future cosmonauts should naturally belong to the first group and only in exceptional cases to the third. Therefore, training flights on aircraft along Kepler's trajectory not only help to familiarize the men with spaceflight conditions but reveal the individual nature of the physiological reactions and capacity for adaptation.

Due to the brevity of the action of experimental weightlessness, there is no point in planning an extended series of flights because adaptation would not necessarily mean that the individual is capable of remaining a long time /13 under the conditions of dynamic weightlessness encountered in spaceflight.

Prolonged confinement in a soundproof chamber with marked limitation of external information provides insight into the nervous-mental makeup of an individual. Without going into details on the methods of investigation (this would require a special report), we wish merely to say that this kind of testing reveals a man's capacity for accurate performance of an assignment under abnormal conditions. Through it one can trace the daily rhythm of the physiological processes, evaluate the performance of experimental tasks, and, by including stimuli of sudden and strong action, observe the capacity for rapid switching from sleep to activity and vice versa, investigate memory, attention, etc.

From the data on emotional reactions and information obtained during execution of various portions of the training program that greatly affect the human mind, one can draw more or less valid conclusions on the nervous-mental status of the cosmonaut and predict his behavior under spaceflight conditions.

Testing in a heat chamber may uncover latent pathological conditions and individual peculiarities of the reaction to stress. It should also be useful in determining the extent of any increase in resistance to pronounced overheating.

There are just a few published reports dealing with the effect on the organism of accelerations varying in direction and duration of action. Some of them mention the possibility of increasing specific resistance to acceleration by rotation on a centrifuge. The subject is important because of the intense acceleration upon the launching of a spacecraft and on its entering into orbit and later into the dense layers of the atmosphere in the course of returning to the Earth. Hence, centrifuge exercises occupy a major part of the special training program. Their purpose is to familiarize the cosmonaut with the accelerations he is likely to encounter in spaceflight (in intensity and duration), increase resistance, and determine individual tolerance /14 of the factor. The cosmonaut is subjected to a series of actions gradually increasing in intensity and duration. The exercises should end with a test of his tolerance of accelerations similar to those in actual flight. They also provide a good opportunity for very objective and scientific evaluation of matters pertaining to potential disability. We wish to stress the importance of careful observation of the cosmonaut's condition during this phase of the program because the available information on the possible cumulative effects of acceleration is conflicting and we do not have a scientific basis for devising rational training exercises. The flights of our cosmonauts have confirmed the soundness of using the centrifuge. Every man mentioned its importance in his report.

Training in a model of a spacecraft cabin and trainer is essential. In them the cosmonaut works out and perfects the flight plan, develops skill in controlling the craft and its onboard systems, and learns how to handle

himself properly in emergency situations. The value of this training is seen in the fact that until he is actually in space, the cosmonaut has no way of increasing his skill in flying with an instructor, as an aircraft pilot or automobile driver can do. Thus, space trainers should enable the future cosmonaut to acquire the skills he will need in space. Since the flight plans and assignments keep changing, the training programs should be modified accordingly.

The experience gained in supervising the health of G. S. Titov resulted in a re-examination of the preparation and special vestibular exercises designed for the cosmonauts. We cannot go into all the theoretical considerations that may account for the mechanism of the autonomic disturbances noted in Titov. We need only say here that special vestibular exercises were devised to prevent such phenomena in the subsequent flights. Use was made of a variety of methods for determining the idiosyncrasies of vestibular function when the visual and motor analyzers are affected. Tests were also made of vestibular reactions to angular, linear, and Coriolis accelerations and of the possibility of inhibiting these reactions through vision and muscular exertion. /15

The special training program aims at increasing vestibular resistance to external influences in the most varied of circumstances, strengthening of the interacting system of vestibular, visual, and motor analyzers to overcome impairment of spatial orientation under the conditions of altered gravity, and reinforcing inhibitory influences on vestibular function. Exercises must be tailored for each cosmonaut with emphasis on the weakest link in his vestibular system. Improved methods of testing this function can reveal the weak links by determining the thresholds of sensitivity of the semicircular canals and otoliths to adequate (angular and linear) accelerations and to inadequate (electric current) stimulation. These testing methods, systematically used in the course of training, can be used to evaluate the reliability and effectiveness of the program.

The overall purpose of the methods is to discover the sensitivity of the vestibular apparatus following exposure to a variety of visual stimuli and muscular exertions. They will provide some idea of how the vestibular apparatus of a cosmonaut will respond to change in visual or motor function, an obviously important matter for spaceflight.

The various special exercises should be supplemented by general physical conditioning aimed both at increasing strength and at improving the visual and motor skills that are so important in spaceflight--bodily control in space, coordination of movements, strengthening of volitional qualities, increase in nervous-emotional stability.

The special training program should allow for individual differences, thereby greatly facilitating the task of evaluating each person's tolerance for various loads. The sequence, alternation, and intervals between the different kinds of tests and exercises are also important.

Thus, the combined measures designed for the biomedical preparation of the cosmonauts help to improve the physiological systems of the body through exposure to spaceflight factors simulated in the laboratory to ensure the rapid reorganization of these systems in case of emergencies. They also promote a favorable nervous-emotional background and strengthen volitional qualities.

EXPERIMENTAL PSYCHOLOGICAL INVESTIGATION OF COSMONAUT TEAMS

F. D. Gorbov and M. A. Novikov

Experimental psychological studies are at the present time an /17
integral part of investigations in the method of selecting and training cosmonauts. The new science of space psychology which has arisen during the last few years makes use of the achievements of general psychology and human engineering, the science of higher nervous activity in man, cases in aviation medicine and psychology, and, in particular, medical flight examinations. Aside from this, it has its own subjects and methods of investigation. The historical flights of Soviet cosmonauts demonstrated the place and significance of psychological investigations during the cosmonaut's training period and during flight.

Up to the moment when we started the investigation of the future cosmonauts there were still no definitive or selective characteristics of a cosmonaut's profession, no so-called professiogram, i.e., no basic foundation on which to establish teleological (end-directed) methods of selection and training. For this reason we isolated those factors of flight activity which could be related to the cosmonaut's profession.

The following are the factors which could be thus related.

1. Continuity of activity. Even when automatic devices, e.g., an autopilot, are present, and when it is possible for the pilot to disengage himself from direct control of the vehicle, all of the most important periods of flight demand continuous attention.

2. Compulsory or constant sequence of operations. We refer here to a compulsory sequence of operations according to a definite program. No changes in the sequence of the cosmonaut's operations can be tolerated; at the same time the duration of each operation is strictly observed.

3. The factors of continuity and compulsory sequence of working operations are also represented in industry (e.g., in conveyors), in transportation and in sports.

4. Scarcity of time. The independent importance of this factor in /18
flight operations was first pointed out by V. S. Alyakrinskiy. The scarcity of time, or time limit, restricts the majority of operations in flight, as well as the reception and processing of information and the pickup of radio signals and communications.

5. The factor of the extension of functions deals with the inability of a man in flight to observe directly the result of his efforts, of his activity. The useful effect or result of an operation is perceived indirectly. We may tentatively speak of the "extension" of functions, or of the "extension" of the sense organs. Thus, the vocal-auditory function is "extended" by transmitting and receiving radio equipment, the visual function by optics, the kinesthetic function by the controls and the sense organs by instruments (thus, instrument indicators are inserted in the functional circuit of reverse afferentation). The man must even depend on the readings of instruments for information concerning the equilibrium of his own body. Thus, the factor of the "extension" of the functions must be recognized as real and substantial in effect.

6. The posture factor combines such effects as changes in the position of the support area, changes in the pressure on the support area during G-loads, absence of a support area (during weightlessness) and loss of the support area, which occurs as a side effect during certain maneuvers and is spontaneous, as for example in parachute jumping.

Associated with the posture factor is the development of neuropsychic stresses (survival, collapse, falling) and impairment of the perception of space (illusions). The posture factor is invariably present when a man is actively steering a vehicle moving through space at a high speed. Therefore, in discussing the posture factor we distinguish the tasks of developing the necessary postural skills and of achieving a sufficiently high level of psychological, muscle-tone and postural adaptation.

7. The "novelty" factor is attended by great emotional stress during the waiting period when visualizing events, as well as by nervous and mental stresses associated with orientational and operational activity under new conditions.

8. The factor of changed (reduced) afferentation. First of all, a word should be said about the total lack of afferentation. It is believed that a lack of total afferentation may develop during spaceflight as a result of weightlessness and the possible limitation of radio traffic with Earth in one-man flights. A further word is also necessary concerning the changed conditions for the reception of signals against a background of noise. During flight according to a definite program visualization patterns are easily set up, and in the absence of adequate means of transmitting corrective and prompting commands difficult situations for the nervous system arise. In cases where signals begin to bear a monotonous character, they easily acquire the /19 characteristics of a summation stimulus, which, if continued, leads to such undesirable consequences as overfatigue and cumulative explosiveness.

Analysis of the difficult conditions arising during flight has shown the significance of the factor of changed afferentation.

The problems of information during flight (in a narrow sense, signaling, and in a broad sense, general afferentation) are connected with the energy-adaptation aspect of information, besides which there is also the human-engineering aspect of the problem, associated with the code-sense aspect of information.

A discussion of the cosmonaut's professiogram would not be complete without mentioning the new relationships which develop during spaceflight between operational activities (in the generally accepted sense) and everything else which occupies the cosmonauts over and above such activities. Food and water intake, physiological functions, moving about in the cabin are unrelated to the task of steering, as are rest and sleep, and were all the subjects of investigation during the first spaceflights. In this respect they constituted elements of the operational activity and consequently fell within the scope of space psychology.

In the aggregate, the indicated factors bear a direct relationship to the problem of the psychophysiology of speed. It is important to note that all these factors came to be known as a result of investigating those operational conditions which arise in the case of persons inadequately trained to cope with the indicated factors. Because of the fact that enormous distances in space are covered in a short time, events which are ordinarily unnoticed and unmeaningful are converted into noticeable, meaningful events. Thus, at high speeds there occur short-duration lapses of memory "on the go," and sometimes difficult situations arise in the form of short disruptions or derangements, first described by S. N. Davidenkov.

A knowledge of the difficulty and effect of psychological factors has also made it possible to determine the trend of experimental psychological investigations carried out with the aim of establishing which persons are unfit for spaceflight and of further toughening those candidates shown to be fit.

The fact is that only those candidates can be regarded as acceptable for flight who are not only in good health, but who have also displayed a high level of functional adaptability of the organism to the known and probable factors of future spaceflight activities.

Because of this consideration, the functional test has acquired primary importance. Functional tests have been instrumental in revealing even latent pathology, making it possible to bring to light and reject persons with subtle psychopathological peculiarities.

For this purpose advantage was taken of the experience gained in /20
medical flight examinations of flight personnel in investigating so-called inaccessible situations (for example, aircraft accidents), which are impossible or dangerous to duplicate.

The necessity for retrospective evaluation of situations developing in the past has led to the development of a whole series of experimental test stresses, simulating or reproducing various flight factors. Table 1 lists the tests which were applied and indicates the principal operating factors of the test and the factors of actual flight which correspond to them.

Tables 1 and 2 reveal the role of similarity or analogy between the effect of the test and a given factor acting on the human organism during flight.

TABLE 1. METHODS OF INVESTIGATING NEUROPSYCHIC STABILITY BASED ON THE REPRODUCTION PRINCIPLE.

Name of test	Basic operating factor	Flight activity factor	Tentative designation of reduced tolerance syndrome
Pressure chamber test	Change in respiratory metabolism	-	Reduced (poor) tolerance to hypoxia
Ascent to 5,000 m, respiration under excess pressure	Excess pressure of O_2 in inhalation and exhalation		Reduced tolerance to respiration under excess pressure
			Decompression disturbances
Ascent to 1,200 m	Decompression factor	-	Impairment of barofunction of ear, meteorism
Period of pressure	Change in pressure of gas contained in body cavities		
Centrifuging	Radial acceleration. Postural and vestibular factor	Piloting	Reduced tolerance to G loads (in units)
Barany chair test	Angular acceleration. Postural and vestibular factor	Piloting	Impairment of vestibular-vegetative sensitivity
Khilov swing test	Summation vestibular stimulus. Posture factor	-	Impairment of vestibular-vegetative sensitivity
Spinning chair test	Posture factor	Arbitrary change in direction or effective G loads	Vascular-dystonic symptoms. Increased muscle tension
Long-duration standing test	Posture factor		Vascular-dystonic symptoms

The first step in this work was the isolation of the factors of psychological well-being and the development of suitable methods of investigation. /21
During the course of this work we formulated the "reproduction principle" as the methodological basis of our experimental psychological investigation. In table 2 we present some results of our work.

TABLE 2. METHODS OF INVESTIGATING NEUROPSYCHIC STABILITY BASED ON THE REPRODUCTION PRINCIPLE.

Name of test	Nature of difficulty during flight	Factor operating during experiment	Tentative designation of reduced tolerance syndrome
Simulated ascent in pressure chamber	Demarcation of real and imaginary danger	Imaginary and actual hypoxia	Emotional instability. Heightened suggestibility
Investigation of stability of assigned activity to action of noise stimuli	Reception of signaled information under conditions of time limit	Test of ability to interpret partially jammed signal	Lowered noise resistance
Memory test	Reception of signaled information under conditions of traveling great distances in space at high speeds	Consecutive and continuous activity, with alternating operation of effective and trace stimuli	Reduced memory
Posture test Spinning chair	Instability, loss of support area, collapse	Change of position on spinning chair	False perception of spatial positions
Centrifuge		Accéleration and deceleration on centrifuge	Emotional instability (with feeling of horror, fear of falling).
Vestibulometric tests	Maneuver of aircraft		
Reproduction of episodic events in associative speech and object tests	Various episodic accident situations	Free association experiment. Simulation of situations	Selective fixed reactions

The "reproduction principle," as the table shows, can be used in connection with a previously formulated program; at other times it emerges spontaneously, so to speak, and the situation developing in the given case is deduced by simple observation.

The metnods of experimental investigation indicated in table 2 have been, in part, published and put into practice. For this reason we shall dwell on only one of the areas of investigation, namely, suggestibility. /22
Clinically, this term has a very specific meaning. The phenomenon of suggestibility is encountered fairly frequently even in healthy individuals in everyday

situations, at home and at work. Nevertheless, the objective study of suggestibility, despite the practical importance of this personality trait, has thus far received inadequate attention. For the insufficiently experienced man, the carrying-out of new and important tasks is attended by considerable neuropsychic stresses, both during the training period and the period before lift-off. A high level of the functional resources, in the neuropsychic sphere, of a man who is in a stressed state of expectation is reflected in his ability to concentrate, to master his mounting anxiety in an orderly manner and to cope with feelings of diminished self-confidence and apprehension concerning a favorable outcome. On the contrary, a reduction of the functional resources is manifested in heightened anxiety and lack of self-confidence, in that intricate yet subtle complex of symptoms usually known as apprehension or heightened suggestibility.

Extreme apprehension and heightened suggestibility can occur under the conditions of prolonged actual flight, when a man finds himself isolated from ordinary happenings, in solitude, when an urgent need arises for demarcation of real and imaginary dangers.

In this connection we quote the words of pilot-cosmonaut A. G. Nikolayev: "It's an interesting phenomenon when it starts to burn--you hear a snap. You wonder whether a piece of the skin has broken loose from the spacecraft. But I know the design of the ship, and I could never have such doubts. I say to myself, 'Calm down, let it burn--it's a normal descent.'" (Pravda, No. 234, 22 August 1962.) This statement is evidence of the great self-possession of the cosmonaut, and at the same time it depicts one of those situations when an evaluation of the current moment is made by repressing natural anxiety by intellectual and volitional efforts.

Experimental investigation of suggestibility is based on the application of electrophysiological methods with the aim of establishing individual characteristics on the basis of the adoption of a rhythm (rhythmic photostimulation, stimulation by M. N. Livanov's method, trigger stimulation) in combination with certain methods of hypnopedia. In addition, we also used word-pair experiments, making it possible to distinguish "leader" and "led." The use of the Rorschach test is of secondary importance, as are pharmacological tests with the application of placebos (A. Ye. Uglov) together with media having a definite and single value.

A discussion of the psychological characteristics of group activity is especially important. The fact is that psychologists have long been interested in studying the cooperation between people under difficult conditions. /23 Formerly the discussion centered on long expeditions and overwintering. At the present time the interest in group psychology is connected with protracted spaceflights. Here, as in no other field of group activity, the requirements for compatibility, cooperation and, of course, a wise choice of personnel are very great, since the members of the group will be spending all their working and leisure hours together. Under these conditions, any disagreement or incompatibility, no matter how slight, can develop into a conflict with all its consequences.

Cooperation is a basic element of sports activity, where the factors of harmony and teamwork are of decisive significance. Experience has shown that even in cases where an excellent personal character has been demonstrated in situations extraneous to group activity, such a character, in a nongroup situation, has only a relative value for determining what contribution would be made by the person in question during a period of cooperative group activity.

Consequently, harmony and teamwork appear to us to be basic concepts in defining that quality of a group which may tentatively be designated as the cohesiveness of the group. A cardinal concept is that of strategy, both as regards the general conduct of the group as a whole and the behavior of the individual operator.

When we began this work we had at our disposal only individual works of Bekhterev and Myasishchev, who, however, did not have any methodological accomplishments to their credit. The concepts of Moreno and other sociometric workers seemed reactionary to us, while the formulations appeared foggy and at times mystifying (ill-famed "bodies"). The studies of Kurt Levin on group dynamics were more helpful, but the experiment itself, based on the hierarchical principle which is predetermined and fixed by subordination of one member to another, was also not useful to us.

Proceeding from this, we formulated two basic areas of investigation:

(1) the study of the psychological aspects of cooperation of individuals in the process of interdependent activity;

(2) the study of the psychological aspects of living together under the complex conditions of protracted group isolation.

Each of the stated areas has its own specific characteristics, but in addition there are many related features which make it possible to advance some overall methodological principles: the principle of psychophysiological compatibility; the principle of functional coordination (i.e., the principle of the subordinative and psychological interdependence of the subjects); and the principle of integrative evaluation of the activity of the group.

Psychophysiological compatibility is an important condition for effective group activity. Recognizing that the basis of compatibility is a community of aims, interests and needs, as well as the absence of any pronounced egocentric aspirations in the group, we cannot ignore the effect that people have /24 on each other under conditions of close contact and collaboration. A significant role is played here by intersuggestibility, interinduction, imitation and mutual syntonicity.

Under experimental conditions a basis for observations is furnished by imposed speech and behavioral reactions and by emotional and psychic manifestations (indicative movements, speech, mimicking, vegetative changes). It is characteristic that under conditions of compatibility we observe a coincidence of the dynamic trend of both external behavioral reactions and physiological characteristics during periods of joint activity and in emotional reactions.

We chose the word-pair test developed by M. A. Novikov from among the methods directed at determining psychophysiological compatibility.

In coordinated work it is possible to observe not only an adoption of the response reactions of one examinee by the others (which frequently make it possible to detect a leader), but also a coincidence of the dynamic trend of pulse variations (processed by the sliding-interval method) with a high coefficient of correlation (0.65).

In uncoordinated work we note an absence of behavior mimicking and a low coefficient of correlation of vegetative reactions.

The homeostatic method was developed for studying integrative group activity on the basis of observations made in a shower room, designed for simultaneous use by four people, located in one of the hospitals of Moscow.

In this shower room the water was supplied through pipes in series, with reduced optimum levels of pressure and temperature. When the four people entered the room together, it was possible to observe a series of strategics in the behavior of each, designed to create conditions of maximum comfort.

This attempt on the part of one bather to achieve the best conditions for himself quickly led to a reaction on the part of the other bathers, as a result of which the first bather received an excess of either hot or cold water. Only at the price of refraining from egocentric behavior was it possible to work out a set of conditions acceptable to all, which required a "conflict" of strategies in the group situation.

Experimental duplication of a situation approximating the "shower situation" required, first of all, development of an instrument consisting of three or four interdependent devices, connected by equivalent effects, and second, an instrument that would permit presentation of tasks of various difficulty. Thanks to the efforts of Prof. Ye. S. Venttsel' and I. Ye. Tsibulevskiy, an experimental model of this device was developed; Tsibulevskiy later developed the homeostat. Experiments of the first period consisted of investigations of a group of three subjects (different subjects at different times).

In an experimental situation involving the cooperation of two or more /25
people, cooperation having to do with purely human activity, the peculiarities of the behavior and reactions of the examinees are revealed much more fully than in experiments based on the usual scheme "man, signaling device" (combination of stimuli). The design of the instrument makes it possible to carry out independent registration of the performance of each of the subjects and to make performance ratings during the period of investigation; these conditions provided an opportunity to detect and trace the development of individual strategies.

Data characterizing the activity of each of the subjects can be compared with the available biographical (anamnestic) information and the results of special investigations (typological peculiarities, etc.).

Problems of various complexity were assigned, ranging from the simplest, which were solved in a matter of seconds, to those of considerable complexity, whose solution was not always within the capability even of a trained group. The examinees learned in all cases to solve the problems, but they were unable to explain by what process this learning took place. In cases where it was impossible for a given group of subjects to solve a problem, a method was used whereby one of the subjects was replaced by a research worker well acquainted with the operation of the homeostat. It is important to emphasize that after solution of the problem assigned to the new group consisting of two subjects and the research worker, the problem could be solved after the return of the first subject who had been removed because of his inaptitude. How this "transfer" of instruction from two subjects to the third takes place in such cases is still not clear, but the fact that it does take place is of definite interest in connection with the problem of trainability (A. I. Leont'yev).

In the solution of integrative problems, the choice of individual strategies reflects the subordinative and psychological interdependence of the subjects in a specific group, the functional coordination inside the group.

Even the successful solution of a problem is as a rule accompanied by dissimilar personal contributions from the individual subjects. It is nearly always possible to distinguish a subject who is directing the overall strategy of the group, whom one may provisionally call the "leader," while at the same time the remaining partners involuntarily, and often without realizing it, subordinate themselves to his leadership. Apparently, functional coordination is an indispensable condition in the solution of group problems, but it should not be supposed that the "leader" of a group engaged in a given activity will remain the leader in a different specific type of activity.

Thus, one must take into account the definite dynamic character of this structure and its dependence on the character of the tasks.

The manifestation of the subordinative and psychological interrelationships of the operators in the group also makes it possible to characterize both the group as whole and the individual members. The procedure adopted by the group in various integrative tests makes it possible to evaluate the stability /26 of these relationships.

This type of interaction of the subjects can be compared with the Sherrington funnel concept or the competition for a final path.

As is known, in the competition for a final path the effector system, in occupying a path, does not completely displace other systems. What occurs is a game-type interaction, in which the relative influence and contribution of each effector system changes at the moment of reaction.

Something similar takes place when, in experiments involving interdependent activity, there is a transition from problems of a given complexity to problems of a different degree of complexity. Here also there is a "competition for the final path" in the sense that each of the subjects, who in a sense are functioning as complex efferent systems, changes his contribution

and his strategy during the period of activity. Here, with complete isolation of the "leader," it is possible to observe a kind of "hypertonia" resulting from the exaggerated and rigid activities of the "motor-adjusted" companion. In problems where the efforts of the "leader" show up mainly in initiative correction, something resembling a "dull paralysis" takes place with separation of a motor-torpid subject.

The analogy which we have presented cannot, of course, be considered complete, and we are not inclined to regard the homeostatic relationship between the various subjects as a model of the interrelations between the pyramidal, extrapyramidal and strictly segmentary effects of the anterior horn of the spinal cord on the effector apparatus of the extremities. Nevertheless, we cannot escape the feeling that in complex systems built on identical interrelationships the immediate effect of the removal of one member will be basically similar.

The investigation of interdependent activity under experimental conditions, such as the extended isolation of a group living and working together, has substantiated the current importance of group psychology.

Such, in brief, is the incomplete program of the experimental psychological investigation of a group of cosmonauts or, rather, the area of investigation.

The preceding remarks, together with a generalization of published data, indicate that the young science of space psychology, based on the achievements of related branches of science, already has to its credit its own fund of scientific assets.

EFFECTS OF EIGHT-HOUR ISOLATION AND HYPOKINESIA ON SEVERAL PHYSIOLOGICAL AND BIOCHEMICAL INDICES IN MAN

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The problem of isolation and hypokinesia has been attracting increasing attention in recent years (refs. 2 and 6). /27 However, we found nothing in the available literature dealing with the effects of brief isolation and hypokinesia on the principal physiological and biochemical indices in man. A study of this subject was the purpose of our investigation.

Experiments were performed on 10 healthy young persons 21-24 years of age. The subjects were kept for 8 hours (in a sitting position) in an airtight room with forced ventilation with atmospheric air. The oxygen content of the room was 20-21 percent; carbon dioxide, 0.01-0.03 percent. The temperature did not exceed 20-22°C and relative humidity was 50-60 percent. The amount of ventilation ensured the removal of the injurious products of human vital activity from the room.

Before and after the experiments we recorded the subjects' EKG in the three standard leads, pulse rate and arterial pressure, and calculated the stroke and minute volumes of blood circulation, peripheral resistance and cardiac index.

The following indices of external respiratory function with the subjects in a sitting position at rest and after physical exercise (15 squats in 30 sec) were also recorded: rate, depth and minute volume, oxygen consumption, oxygen utilization factor, amount of oxygen (in milliliters) consumed in 1 liter of air, vital capacity, volume of forced exhalation in 0.5 sec (as percent of the vital capacity), values of the oxygen and ventilation debts and rate of /28 their liquidation from the restoration factor in 4-5 min (RF_{4-5}). In addition, in four experiments energy consumption was determined during and after physical exercise from the oxygen consumption.

The following tests were used to investigate the mental sphere: test 1 (check of work performance at an excessively fast rate and distribution of attention using a table of numbers and letters) and test 2 ("counting" with switching, to study the formation and alteration of an intellectual skill).

The carboxyhemoglobin content of the blood was determined manometrically in Van Slyke's apparatus, blood catalase activity by A. N. Bakh and R. S.

TABLE 1. EFFECTS OF 8 HOURS OF ISOLATION AND HYPOKINESIA ON SOME INDICES OF THE HEMODYNAMICS.

Index	Direction of changes		
	Increase	Decrease	No change
Pulse rate	1	8	1
Maximum arterial pressure	3	3	4
Minimum arterial pressure	4	2	4
Mean arterial pressure	5	4	1
Pulse arterial pressure	3	5	2
Stroke volume	6	3	1
Minute volume	3	7	-
Peripheral resistance	6	4	-
Cardiac index	3	7	-

Zubkova's method (ref. 1) and serum cholinesterase activity (ref. 3). The urea content of the blood, and ammonia and urea content of the urine (by Conway's method) were also recorded.

Changes in some indices of the hemodynamics are presented in table 1. It is evident from the table that, after 8 hours of isolation and hypokinesia, most of the subjects experienced the following changes in the cardiovascular system: decrease in pulse rate (by 16 percent), slight increase in stroke volume (by 11 percent), decrease in minute volume and increase in peripheral resistance (by 23 percent). EKG changes did not occur except for a tendency to bradycardia.

Table 2 shows that, after the experiments, the changes in external respiratory function were in different directions. However, these changes remained /29 within normal physiological limits, except for a tendency toward a slowing of the rate of forced exhalation in 0.5 sec. After physical exercise, the oxygen debt in most of the subjects was liquidated somewhat more quickly and the ventilation debt more slowly (decrease in the RF_{4-5} from O_2 and increase in the RF_{4-5} from the minute volume of respiration). The expenditure of energy on the physical exercise decreased after the experiment in all four subjects (by an average of 14 percent) tested for this purpose. The decrease resulted from a decrease in the oxygen debt.

Analysis of the results of the psychological tests shows that intellectual efficiency at the start of the experiments was influenced by the emotions of the subjects because of the novelty of the situation. At the end of the experiments, most of the subjects made more errors in test 2. The errors of "switching" were predominant, suggesting some sluggishness of the nervous processes.

TABLE 2. EFFECTS OF 8 HOURS OF ISOLATION AND HYPOKINESIA ON EXTERNAL RESPIRATORY FUNCTION.

Index	Direction of changes		
	Increase	No change	Decrease
Pulse rate	3	2	5
Depth of respiration	4	3	2
Minute respiratory volume	2	5	3
Oxygen consumption	5	1	4
O_2 utilization factor from the RF_{4-5}	4	-	6
from the minute respiratory volume	6	1	2
from O_2	1	1	7
Vital capacity	-	9	1
Forced vital capacity in 0.5 sec, percent	1	2	7
Energy expenditure on work	-	-	4

NOTE. The RF_{4-5} was investigated in 9 subjects; energy expenditure, in 4.

The changes in the biochemical indices after 8 hours of isolation and hypokinesia are summarized in table 3. They represented a statistically significant decrease in blood carboxyhemoglobin. For example, it was 1.48 ± 0.48 before an experiment, but 0.51 ± 0.26 ($P < 0.001$) afterward. Blood catalase activity increased. The difference in the value of the catalase index before and after an experiment was statistically significant ($P < 0.002$).

Serum cholinesterase activity decreased. If the value of its activity /30 before the experiment was 100 percent, it was 91.2 percent ($P < 0.05$) afterward.

The experimental conditions had no significant effect on the blood urea content. However, there was a tendency for the urine ammonia and urea to decrease.

It is fair to conclude from our investigations that 8 hours of isolation and hypokinesia do not induce functional changes in man.

TABLE 3. EFFECTS OF 8 HOURS OF ISOLATION AND HYPOKINESIA ON SOME BIOCHEMICAL INDICES.

Index	Carboxy-hemoglobin content, %	Catalase index	Serum cholinesterase activity, %
Number of observations	6	10	6
M + m			
before the experiment	1.48 ± 0.48	3.62 ± 0.17	100
after the experiment	0.5 ± 0.26	4.42 ± 0.16	92.1 ± 3.1
P	< 0.001	< 0.002	< 0.05

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EFFECT ON THE HUMAN ORGANISM OF PROLONGED CONFINEMENT IN A SMALL HERMETICALLY SEALED CHAMBER

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Retention of a person's work capability during a prolonged stay in /31
a small hermetically sealed chamber is important at the present time because of the increasing length of spaceflights. A solution to this problem requires a wide range of biomedical research on the effects of prolonged isolation and limited movement during a stay of many days in an artificial atmosphere.

The pressure and gaseous composition of the atmosphere of the "Vostok" spacecraft, as is known, was maintained at terrestrial levels. However, the need to find broader technical and biological means of supporting human life in space has raised the question of possible utilization in spacecraft cabins of an artificial atmosphere with lower barometric pressure and different proportions of the constituent gases.

We point out that practically all programs of manned spaceflights in the U.S.A. make use of a cabin pressure equal to 1/3 atmosphere (245 mm Hg). A decrease in pressure makes it possible, without lowering safety requirements, to reduce the weight of the system by using lighter materials. Furthermore, any gas loss through leaks would be kept to a minimum and a rupture of the airtightness of the cabin would cause a considerable decrease in the extent of the drop in pressure.

Several authors have noted that occurrences of aeroembolism may be prevented by decreasing cabin pressure and increasing the partial pressure of oxygen in an artificial atmosphere (ref. 11).

The influence of low barometric pressure in spaceflight has been /32
studied by Soviet and foreign authors.

The literature contains some references to changes in human efficiency during a prolonged stay (7 days) at an elevation of 3,050 m and in an atmosphere containing 55 percent oxygen (ref. 12). The authors point out that towards the end of the experiment the subjects showed less efficiency and symptoms of asthenia. The subjects complained of boredom and felt the need for variety in the day's routine. In the authors' opinion, such behavioral changes are due to the primitive and monotonous character of the tasks at hand. Recommendations were therefore made to present the subjects with more complicated and

diversified assignments, especially those that could creatively stimulate persons under such conditions.

Interesting results were obtained from an experiment involving two fliers who remained for 17 days in a pressure chamber with a low barometric pressure, corresponding to an elevation of 8,420 m (250 mm Hg) and containing pure oxygen (ref. 13). The length of the experiment was determined by the probable time needed to fly around the moon. During this experiment, the subjects were observed to have irritated respiratory tracts and pulmonary tissue. Both lost weight (2.7 kg and 1.4 kg). The author cited an experiment in which the subjects, kept together for 30 days in a small enclosed space, became irritable and hostile to each other despite their close friendship in the past.

The investigations of Soviet authors (refs. 1 and 5) included studies of persons who stayed for 10-15 days in a small airtight cabin with normal pressure, with a rarefied atmosphere (405 mm Hg). The experiments showed that work capability under such conditions tends to decrease toward the end. Changes in the physiological reactions were functionally reversible.

One study of special interest (ref. 6) explored the possibility of staying for an extended time (up to 30 days) at low barometric pressure, corresponding to elevations of 3,000-7,000 m with a partial pressure of oxygen in the inspired air of 150-200 mm Hg.

The results showed that it is entirely possible to remain for 30 days and fully retain work capability. According to the authors, the rarefaction factor within the limits studied (up to 308 mm Hg) is by itself of no biological significance. The results of certain functional tests carried out during the study (lability of the pulse under a physical load, increase in myocardial contraction time, lengthening of the systolic index and systolic indicator, decrease /33 in breath retention time following inspiration, etc.) indicate a substantial weakening of cardiovascular function.

We believe that the factor of duration itself results in establishment of nervous regulation of physiological reactions at a new functional level. This is borne out by the fact that functional changes tend to increase with the duration of the experiment and produce fatigue and decreased work capability.

A rather complete psychophysiological study was carried out abroad involving 30-day experiments at an elevation of 5,486 m and 17-day experiments at an elevation of 10,200 m (ref. 16). The authors conducted the lengthy experiments in a simulated spacecraft cabin containing an artificial atmosphere for the purpose of studying the effect of the "prolonged inactivity, isolation and a rarefied atmosphere devoid of an inert gas."

These experiments disclosed a drop in diastolic pressure, some decrease in the vital capacity of the lungs, an increase in the time required for restoration of the cardiac rate following an orthostatic test and increase in atrioventricular conductivity and lability of the T-wave of the electrocardiogram. The subjects also lost weight (2.8-3.6 kg during the 30-day experiment and 2 kg during the 17-day experiment) and water from the tissues.

In the opinion of the authors, changes in the water balance during the first half of the experiment were due primarily to loss of extracellular water; during the second half changes took place in cellular composition. Despite the water loss, the fat content increased.

Hematological and clinical laboratory tests as well as examination of auditory and visual sensors did not disclose any significant changes. The functional disturbances noted in the cardiovascular system persisted for two months following conclusion of the 30-day experiment.

Prolonged experiments in hermetically sealed cabins carried out in recent years both in the Soviet Union and abroad have yielded valuable factual material on the work capability of persons confined for many days to small sealed chambers. They have also contributed to the formulation of principles governing the creation of an artificial living environment.

In regard to spacecraft cabins, this problem has its peculiar features due to the special conditions of spaceflight on the one hand, and, on the other, to the existence of a certain compromise between the desire to establish optimum comfort conditions for crew members and the technical means of realizing them. /34

In the opinion of a number of authors, at the present stage of astronautics, the limits of permissible fluctuations in the parameters of an artificial environment should be determined in relation to specific spacecraft and flight programs. It is necessary to take into account the fact that in itself an increase in the duration of spaceflight requires a stricter approach to the establishment of parameters for the environment and a more precise evaluation of the role played by the astronaut as a biological element in the artificial environment of a hermetically sealed cabin.

Simulation of terrestrial conditions may appear to be a simple and rather tempting way to solve the problem, but it is not feasible, principally for technical reasons.

In this connection, physicians and biologists are now faced with the problem of determining optimum physiologic-hygienic criteria for normalizing an artificial living environment and developing an effective work and rest regime applicable to spaceflights of varying duration. This includes determining the limits of permissible partial pressure of oxygen and carbon dioxide, the biological role of inert gases, permissible limits of temperature, humidity, barometric pressure, etc.

The solution of these problems must stem from the premise that in spaceflights, especially long ones, no compromise will be possible at the expense of the human element.

In our work, we studied the character of change in basic functions after prolonged confines of two subjects (more than 30 days) in a small enclosed chamber. The investigation was carried out in a pressure chamber with a volume

of 7 m^3, which was specially equipped for conducting extended experiments. The gaseous composition of the air, temperature and humidity were maintained automatically at a constant level with the aid of a specially developed life-support system (G. I. Badikov, B. A. Miloslavov and G. I. Solov'yev). Throughout the course of the experiment the partial pressure of oxygen remained at 155-165 mm Hg, CO_2 content no higher than 2 mm Hg, air temperature at 19.5-23.5°C and relative humidity at 40-70 percent. Telephonic communication with the subjects was reduced to a minimum.

Throughout the experiment examinations were made of the state of higher nervous activity, bioelectric activity of the cerebral cortex (EEG and electroencephaloscopy), EKG using the three standard leads at rest and after a physical load, arterial pressure, gas exchange, external respiratory function, and saturation of blood with oxygen. Once a week biochemical analyses were made of the urine and blood morphology.

Before and after the experiment, the subjects were given a comprehensive clinical physiological examination, including special functional tests /35 (treadmill test, determination in a pressure chamber of tolerance of moderate hypoxia, rotation on a centrifuge to determine resistance to acceleration, etc.).

Tracings of the physiological functions were made on a multichannel ink-writing oscillograph.

A method was developed to study higher nervous activity in which the subjects responded to various verbal and light stimuli with a complicated motor reaction involving in some cases outlining of geometric figures and in others solving similar arithmetic problems. A special electrocontact pencil was employed to record the motor reactions.

The conditioned reflex method made it possible to study both the latent period and the duration of the motor reactions and also the delicate coordination movements involved in outlining geometric figures. Inadequate reactions were interpreted as impairment of differentiation.

Mental capacity was determined by the speed and accuracy of solving arithmetic problems.

Simultaneously with the motor reactions, we also recorded an EEG, electromyogram of the muscles of the right forearm, electrocardiogram, respiratory movements of the thorax and arterial pressure. These made it possible to determine the nature of the changes in the autonomic components of conditioned reflex activity.

As a result of the aforesaid investigations it was possible to note a wavelike character and phasic quality in the functioning of the higher portions of the brain. Analogous changes were observed during the 10-day experiment (ref. 7). Quite characteristic was the fact that, as the experiment increased in duration, there was an increase in the time of the conditioned reflex elicited by outlining the geometric figure (fig. 1).

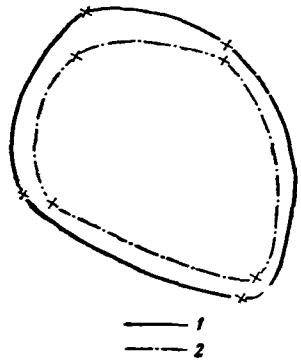

Figure 1. Time needed to outline geometric figure during different days of experiment (subject N). 1, prior to long experiment; 2, after long experiment.

During the first few days of the experiment it took 15-20 seconds to outline the geometric figure, but after 30 days, 25-28 sec were needed, and by the end of the experiment 35-37 sec. The quality of coordination meanwhile did /36 not undergo any substantial change, but it deteriorated during the terminal days of the experiment.

Studies of conditioned activity showed that in prolonged hypokinesia and relative isolation the depth and degree of change in motor functions (time required to outline a geometric figure) were considerably greater than in intellectual efficiency (time required for and accuracy in solving arithmetic problems).

Alteration of the dynamic stereotype with respect to sequence of stimuli and signalling significance resulted in marked lengthening of the latent periods of the reactions. However, the tests with alteration of the stereotype at one end of the experiment were attended by major difficulties, as shown by the increased number of mistakes, by cases of impaired differentiation, etc.

These observations indicate that a prolonged stay by human beings in a small enclosed chamber leads to development of protective inhibition and to decreased mobility of the nervous processes, i.e., their functional inertness.

This is also corroborated by data obtained from observing the behavior of investigators and by analysis of their comments in the logbook. Characteristic reactions included loss of interest by the examinees in the investigations, especially in the second half of an experiment, development of irritability, annoyance, indifference to surroundings, weakening of critical attitude toward their own actions and increasing emotional lability against a background of marked decrease in motor activity.

During the second half of the experiment, the subjects failed to carry out the tasks called for by the program with the desired promptness and accuracy. At the end of the experiment, the subjects had to be constantly reminded of the necessity for carrying out physiological investigations. By the end of the experiment, the volume of physical exercises had decreased considerably. Execution of the physical exercise program, which to some extent made up for lack of motor activity, produced dyspnea, tachycardia, increased perspiration and fatigue.

These facts show that confinement in a small chamber for an extended period of time under the conditons of hypokinesia and decreased information flow from the outside weakens internal inhibition and decreases general excitability and central nervous system tone.

T. T. Dzhamgarov (ref. 3) points out that mental productivity increases despite the oppressive effect of isolation. The changes in higher nervous activity noted during the course of a 10-day experiment, due to the exercising of conditioned reflexes, seem to have been misconstrued by the author as being a specific reaction to isolation and hypokinesia. The decrease in the /37 subjects' mental efficiency during the second half of the experiment can be ascribed to the limited motor activity and a lower level of afferent impulses rather than to "decrease in emotional-volitional tone" (ref. 4).

Decreased tone of the cerebral cortex in our subjects is corroborated by analysis of EEG and electroencephaloscopic findings. Toward the end of the experiment under conditions of relative isolation and hypodynamia, the EEG showed an abrupt drop in the α-index, an increasing predominance of slow waves at a frequency of 4-6 cps, and the appearance of waves in a frequency band of 0.5 to 2 cps.

The progressive increase in intensity of the slow waves may be explained primarily by long restricted mobility and some decrease in the afferent impulses. Confirmation of this is to be found in other and shorter experiments.

It was found that even during the first 10 days of hypokinesia isolated slow waves in the frequency band of 2 to 6 cps appeared in the subjects; these were quite pronounced and rapidly increased in number. By the end of the 30-day experiment they dominated the EEG.

The experiments of F. D. Gorbov and Yu. A. Izosimov involving prolonged complete isolation and hypokinesia disclosed the regular appearance of slow diffuse waves on the EEG of the subjects. The same thing was observed by D. I. Ivanov and V. L. Popkov, while conducting a 30-day altitude experiment in a pressure chamber.

This indicates that prolonged restricted mobility and decreased flow of external stimuli markedly reduce the lability of nerve cells of the cerebral cortex and change the relations between the fundamental nervous processes in the direction of relative predominance of inhibition.

This is also borne out by the conversion of a lively diffuse-focal mosaic of cortical bioelectric activity (according to electroencephaloscopic data obtained prior to the experiment) into a diffuse, immobile one at the end of the experiment. This was accompanied by the appearance of a special form of bioelectric activity known as "overflow," and weakened reactivity to light and sound stimuli, etc.

Evidently the changes in various physiological functions, especially the autonomic, are of central origin in their mechanism and are caused by general decrease in the excitability of the central nervous system. This thesis is confirmed by an investigation of the cardiovascular system and external /38
respiratory function during prolonged hypodynamia and isolation.

Experimental findings showed that toward the end of a stay in a small enclosed chamber the cardiac ratio increased on the average by 8-10 beats or 20 percent of the original rate. These changes were more or less cyclical and wavelike, comparable to those found in studies of higher nervous activity. The arterial pressure of both subjects showed a maximum (10-16 percent) and a minimum decrease (7-8 percent) toward the end of the experiment. This points to a decrease in vascular tone and to a lowering of cardiovascular tone. Toward the end of the experiment, respiration markedly slowed, but oxygen saturation of the blood did not change at all throughout the investigation.

Data on changes in the pulse and respiratory rates and blood pressure are presented in the following table (mean data) for each 10 days of the experiment.

Acceleration of the pulse in hypokinesia may be partially explained by interference with the cholinergic action of the vagus nerve, as occurs in the

Day of experiment	Pulse, beats per min		Rate of respiration per min		Blood pressure, mm Hg	
	Subjects					
	N	Ts	N	Ts	N	Ts
10th	49	66	15	10	113/53	115/60
20th	49	61	15	10	110/50	109/53
30th	58	69	12	9	109/47	110/50
40th	56	65	10	8	104/50	110/51
50th	56	73	10	9	105/48	109/51
60th	59	74	9	8	107/48	105/51

absence of necessary physical activity. The predominance under such conditions of the adrenergic action of the sympathetic nerve results in metabolic changes in the blood vessels and tissues.

The foregoing is applicable to the condition of the heart muscle. Cardiac activity plays a major role in maintaining the blood pressure level since a decrease in the cardiac output or stroke volume lowers blood pressure.

We believe that the reason for the drop in blood pressure of our subjects is the change in the interrelation of cardiac activity and vascular tone stemming from restricted motor activity. /39

Clinical examination of the subjects after a prolonged experiment also showed a considerable change in hemodynamics (N. V. Pisarenko). Observations showed an increase in the stroke volume and cardiac output, rise in the oscillator index and decrease in the ratio of the modulus of elasticity to peripheral resistance. Plethysmography disclosed a lengthening of the latent periods of the vascular reactions, although the reaction time did not stay constant, as shown by the instability of vascular tone.

The marked change in cardiovascular function is attested to by the data obtained when examining the subjects on the treadmill (running for 3 min at 3 m/sec; fig. 2). Prior to the experiment, respiratory and cardiac activity were fully restored to normal 4-5 min after the run was over. When this functional test was taken after termination of the extended experiment, pulse excitability had increased almost 400 percent over the original level and there was a relative lengthening of the electric systole and systolic index, indi- /40 cating a lowering of cardiovascular function. Meanwhile, the time needed for the physiological indices to return to normal increased almost 300 percent (Ye. A. Poruchikov).

The functional tests indicated a considerable decrease in adaptability of body mechanisms to physical exertion and a sharp increase in excitability of the blood circulatory apparatus.

The roentgenokymographic investigations conducted by P. N. Ivanov and A. R. Mansurov are very interesting. They show that a prolonged period of decreased motor activity and isolation caused a significant decrease in the heart size of both subjects, indicating that the heart suffered from a lack of exercise (fig. 3).

An investigation of gas exchange showed that during the course of the experiment oxygen consumption decreased 32 percent on the average, while the exhalation of carbon dioxide decreased 26 percent. Pulmonary ventilation dropped 2-2.5 liters per minute. This reflects changes in the energy requirements of the human organism during prolonged hypodynamia. Initially, energy expenditures amounted to 30.15 large calories per kilogram of weight per day, but by the end of the experiment they had dropped to 20.85 large calories per kilogram per day (I. P. Neumyvakin, Ye. A. Kovalenko and N. S. Mikerova).

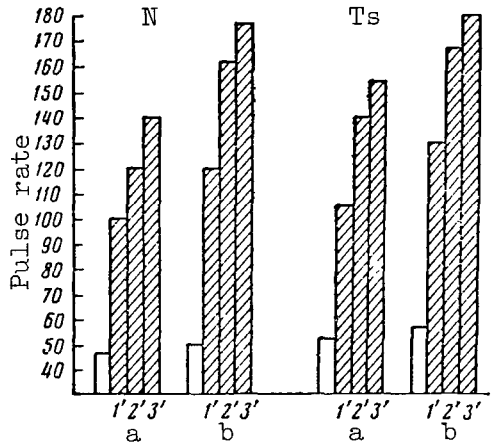

Figure 2. Character of change in the cardiac rate in treadmill tests before and after termination of the experiment: a, before confinement; b, after confinement; white column = before treadmill test; 1' = one min after; 2' = two min after; 3' = three min after.

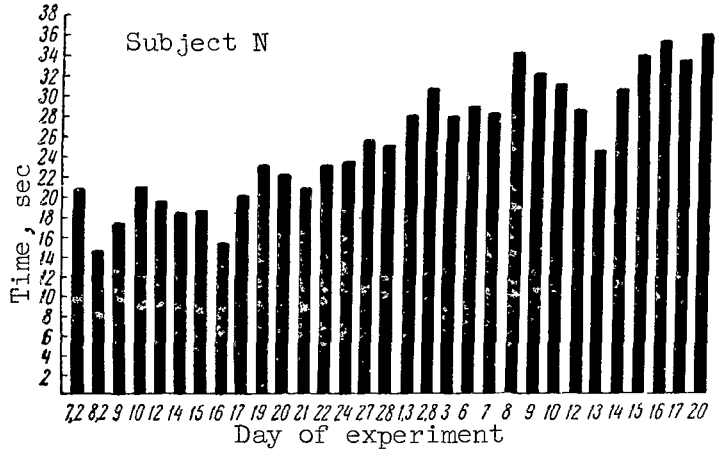

Figure 3. Roentgenokymographic changes in dimensions of heart's "shadow" before and after experiment according to conditioned motor reaction time to red light.

The peripheral blood showed a temporary increase in the number of erythrocytes and reticulocytes. On some days, the lymphocytes also showed an increase. In the second half of the experiment, the absolute number of eosinophils increased 1.5-2 times, indicating a certain functional stress in response to subjection to prolonged hypodynamia and isolation. An investigation of phagocytic activity of the neutrophils showed that it increased toward the end of the experiment. /41

The clinicophysiological studies carried out jointly with N. V. Pisarenko, V. I. Kopanev, Ye. A. Poruchikov and M. A. Cherepakhin prove that the changes resulting from prolonged confinement in a small enclosed space are functional and reversible in character.

Study of the dynamics of restoration of various physiological reactions showed that motor changes persist longer than autonomic changes.

We believe that such changes can be prevented only by doing everything possible to compensate for the lack of motor activity and to provide for the optimum amount of sensory information. An important consideration in this connection would be the development of a scientifically grounded, rational work and rest routine, the strict observance of which would make it possible to tolerate hypokinesia and isolation successfully.

Tests conducted immediately on completion of the prolonged experiment showed that the capacity for physical work and static endurance decreased in both subjects by 16-36 percent, while the capacity to maintain equilibrium under static conditions decreased by 40 percent (M. A. Cherepakhin). Consequently the physical load used in our experiment did not fully compensate for the lack of motor activity. It will be necessary in the future both to increase the physical load and to distribute it effectively over the course of the day. Proper organization of work and regular performance of graduated physical exercises may, to a certain extent, compensate for the unfavorable effect of prolonged hypodynamia and isolation by virtue of the central nervous system receiving an inflow of new strong proprioceptive impulses. A system of planned muscular exertions fulfilling the natural need for motor activity should contribute to the maintenance of normal tone of the central nervous system and produce a favorable effect on the various functional systems.

Stabilization of the basic physiological indices should in its turn exert a favorable effect on maintaining physical fitness and on increasing mental productivity during prolonged spaceflight.

In recent years long-term investigations have been conducted which /42 concentrated on the study of such prolonged spaceflight conditions as restricted mobility, isolation, monotony and the constant threat of external hazards, their effect on interrelations among the crew, etc. (refs. 14 and 15).

Psychological observations and special tests were used to determine the optimum conditions conducive "to maintaining a harmony of temperaments" and normal relations among crew members. In the authors' opinion, spaceflights with two-man crews are not desirable from the psychological point of view. Long-term experiments on isolation showed that certain habits, character traits and even such trifles as noisy eating, untidiness, etc. played a part in influencing relations among the subjects. The factors tended to irritate even those who had previously considered themselves to be the best of friends.

A crew of three members is likewise unstable, according to the authors, because of the possibility of two members ganging up on the third.

The preparation of prolonged spaceflights and the organization of a work and rest routine require, along with the previously enumerated physiological and hygienic measures, that everything possible be done to overcome monotony (monotony in the cabin's interior, nature of duties, etc.). Monotony has an adverse effect on man and on his productivity, to which considerable attention had been devoted in the early days of machine production (ref. 8). Monotonous work was shown to impoverish the psychological content of work and decrease a person's interest and creative attitude toward the work to be done.

The nature of the effect of monotony was elucidated by I. P. Pavlov's physiological theories (ref. 9). He wrote: "...The concentration of stimuli in one spot or, as we say in the laboratory, chiseling into one cell, causes that cell to become a refractory, inhibited, unexcitable. This condition then spreads over the entire cerebral hemispheres and results in sleep."

Our observations show that different subjects spending a long time in a small enclosed chamber react differently to a monotonous setting under identical conditions. This factor must, therefore, be given very serious attention in the selection and training of cosmonauts.

Also of importance are not only a simple quantitative increase in the flow of afferent information, but proper organization of the work and rest routines: efficient sequence of tasks, introduction of more creative work and variety of physical exercises, intelligent use of music, radio communication and television. In other words, it is necessary to create "a stimulating psychological atmosphere."

Other important factors include personal interest on the part of the subjects, awareness of their obligations as citizens and high moral responsibility for the way in which they carry out their assignment. The last consideration will be especially important in carrying out prolonged spaceflights.

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REACTION OF THE HUMAN ORGANISM TO IMPACT ACCELERATION STRESS ACTING IN VARIOUS DIRECTIONS

G. P. Mirolyubov

We know that the most effective way for a cosmonaut to return from a /44
long flight is to have him land directly in his spacecraft. The primary advantage of the spacecraft is that it is the most reliable and safest way of returning to Earth or of landing on other planets. However, at the moment of landing, the vehicle undergoes impact acceleration stress which, under unfavorable conditions, may reach sufficient magnitude to affect man adversely.

Problems relating to the study of the effect on man of impact acceleration stress and of methods for easing them have been of timely interest for the past 3-5 years. Papers have been published which deal with the investigation of impact acceleration stress occurring in falls of human beings (refs. 1-4), aircraft and helicopter accidents (refs. 5 and 6), and motor accidents (refs. 7 and 8). A large number of experiments have been carried out by various scientific research centers in the U.S.A., aimed directly at developing ways of landing pilots safely in a spacecraft capsule (refs. 9-14) and in the cabin of a crashing aircraft (refs. 15 and 16). By the use of different shock-absorbing devices (air, honeycomb aluminum, honeycomb paper, etc.), American investigators have succeeded in saving human beings from vertical falls of aircraft cabins at 7-8 m/sec. Nonetheless, excessive impact acceleration stress at different angles, despite careful preparation of the experiment, produced complaints of pain by the subjects and occasional damage to the spinal cord (ref. 17).

Unfavorable cases bear witness to the inconclusive solutions for the problem of safe landing under complicated conditions (lateral wind, uneven landing platform, etc.). Much importance is attached to the manner and mechanisms by which clinicophysiological and traumatic injuries arise (refs. 18 and 19) /45
and to preventive measures (refs. 20 and 21). Intensive research is going on in the U.S.A. on the action of impact acceleration stress occurring at various angles relative to the vertical and horizontal axes of a person seated in a chair (refs. 22 and 23).

The present study presents the data of experiments on impact acceleration stresses similar to those incurred in landing.

The investigations were conducted with ground-based apparatus specially equipped for studying methods of protecting crews of aircraft in forced landings and the passengers of motor vehicles and other forms of transport in accidents. The subjects were put in a reinforced mockup of a cabin.

The impact acceleration stress was created by braking the cabin with a shock absorber after it had been accelerated to the required speed. The present experiments utilized two designs of a pneumatic shock absorber (Pn-1 and Pn-2) and one mechanical absorber (Mech).

The type and design of the shock absorber determined the character of impact acceleration stress. When braking with pneumatic shock absorbers Pn-1 and Pn-2, the recorded acceleration curves were in the form of a polysinusoid, the growth rate of which was 400-500 and 1,000-1,200 units per sec, respectively, while the size depended on the cabin's speed of movement.

When braking with the mechanical shock absorber (Mech), the acceleration curve resembled a trapezoid. The intensity of impact acceleration stress, regardless of the cabin's speed, was 10-12 units for a growth rate of 700-1,000 units per sec.

Recording of impact acceleration stress was achieved with the aid of two types of sensors. Tensometric sensors (MP-01) were attached to the cabin and the subject's head; the recording was made on a K-20-21 oscillograph (range of measured acceleration was ± 30 units, the actual frequency of oscillations 450-900 cps). Two ZP-16 recording instruments were installed in the cabin (the range of measured acceleration in three planes was ± 30 units; actual frequency of oscillations was 70-100 cps).

It was possible to study six angles in the operation of impact acceleration stress by changing the position of the seat of the subjects and the direction of the cabin's motion (fig. 1). Nine series of experiments were conducted in all. The basic data characterizing these experiments are presented in table 1.

The experiments involved the participation of physically healthy subjects, volunteers 23-35 years of age (including one woman). They included physicians, engineers, technicians and sports parachutists.

All subjects underwent roentgenoscopic control examinations. On the /46 day of an experiment, each subject received a medical examination 30 minutes prior to exposure to impact acceleration stress and 20 minutes afterward. In examining the subjects, special attention was paid to their general state of being, residual tenderness in movements of the joints; a functional load test of the cardiovascular system, 20 squats in 20 sec, was given.

The subject's condition was kept under observation from the moment he was seated in the cabin mockup to the moment he left, including the time he was subjected to impact acceleration stress (5-7 min), by recording an EKG, kinetocardiogram, seismocardiogram of the rate of thoracic respiratory movements and the arterial blood pressure. The speed of the subject's motor reactions was verified with arrhythmic photic stimulation.

The subjects felt the impact stress load as a sharp jolt, similar to that experienced when catapulted in a chair or falling on snow with a hard crust. Examination of the subjects after landing showed no damage to the skin. Joint movements were painless and had full scope. The subjects did not complain of

TABLE 1. EFFECT OF IMPACT ACCELERATION STRESS ON THE HUMAN BODY.

Experimental series	Direction of impact acceleration stress (fig. 1)	Type of shock absorber	Maximum speed, km/hr	Maximum impact acceleration stress exerted on cabin			Maximum impact acceleration stress exerted on head of subject			Remarks
				Intensity, in units	Duration, msec	Growth rate, units/sec	Intensity, in units	Duration, msec	Growth rate, units/sec	
1	1	Pn-1	36	27.4	110	400	30.5	110	420	No complaints
2	1	Pn-2	28.8	20.1	70	1,200	31.0*	70	1,200	" "
3	1	Mech	36	11.8	160	1,000	10.5	150	700	" "
4	2	Pn-1	38.7	21.2	150	300	-	-	-	Violent shaking of body
5	4	Pn-1								
6	3	Pn-1	36.0	29.6	110	400	35.2	110	500	Painful sensations in cervical and thoracic sections of spine
7	3	Mech	36.0	13.0	110	1,000	14.2	110	700	No complaints
8	5	Pn-1	25.2	16.2	120	400	23.0	110	450	" "
9	6	Pn-1	25.2	15.8	120	400	25.0	110	460	Rotation of cardiac axis to left, no complaints

*Impact acceleration stress measured on chest.

any deterioration in their sense of well-being. However, the sixth series /48 of experiments was an exception: in two cases the subjects felt pain in the cervical and thoracic segments of the spine on exposure to impact acceleration stress (maximum intensity). Despite the absence of objective data relating to damage to the vertebrae, one subject experienced pain for several days while moving around.

The subjects stated that impact acceleration shock in a longitudinal direction, shown in figure 1, 3) and 4), were felt more severely than lateral ones, shown in figure 1, 1) and 2). One of the reasons for this is tilting of the head and arms forward and downward at the moment of impact and shifting the entire body downward in the direction of the force of the impact. Motion pictures

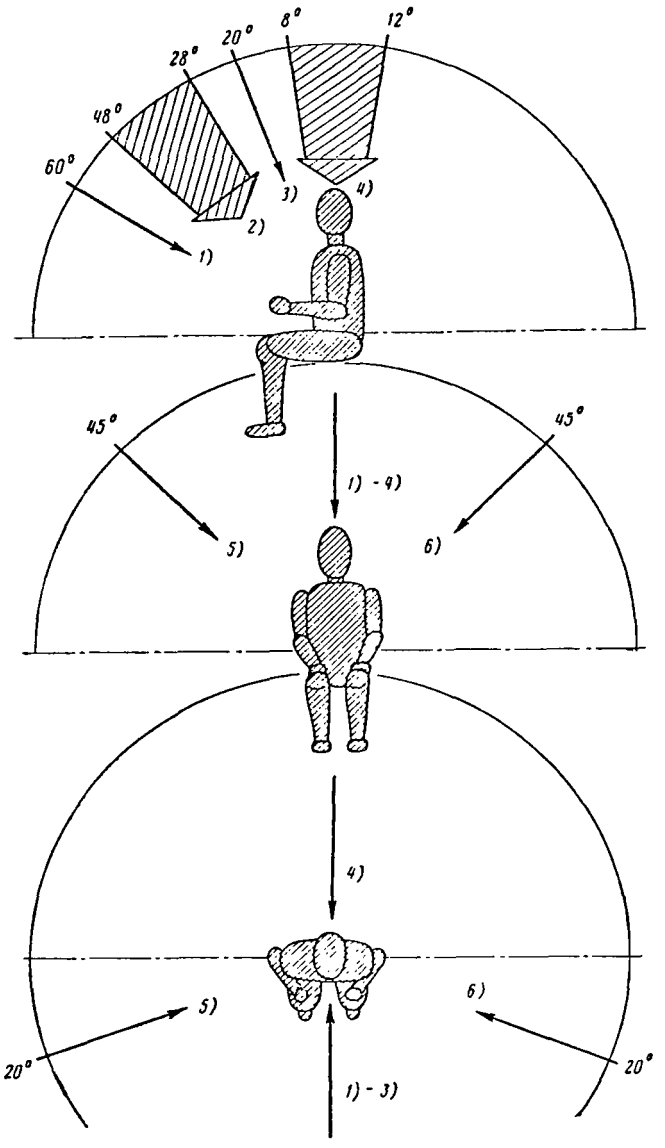

Figure 1. Diagrams representing directions of impact acceleration stress in relation to seated person. 1-6, Directions of stress.

(600-800 frames per sec) showed that the head tilted forward 40 mm, while the shoulder girdle was displaced downward 50-60 mm.

The impact acceleration stress exerted on the head exceeded by 1.5-5 units that recorded on the chair. This difference is directly related to the magnitude of tilt of the head and shifting of the shoulder girdle. This difference, caused by the dynamic reaction of the body, is of practical interest because any

decrease in intensity of acceleration stress experienced by the subject as compared with the chair increases human resistance.

No significant deviations in the functioning of individual organs or systems could be observed 15-20 min after landing. Pulse and respiratory rates, arterial pressure, coordination of movements and other functions of the organism were virtually identical with the findings before exposure. Tests of the cardiovascular system with a measured load, which are regarded as an integral index of the organism's functioning, also attested to preservation of a fairly high level of functional capacity of the myocardium and vascular tone. Before and after exposure, the cardiac rate in response to a physical load increased 20-40 beats per min. After 3 min it returned to the initial level, and in most cases dropped somewhat below (table 2), which is a sign of good function (ref. 24).

The dynamics of arterial pressure in tests with a measured load are presented in table 3. The data show that the maximum arterial pressure increased 10-20 mm Hg in response to the load, both before and after exposure. At the end of 3 min, the maximum arterial pressure dropped and approached the initial figure.

In evaluating the character of the change in maximum arterial pressure, it should be noted that it was slower returning to normal than the pulse rate. According to the literature (ref. 24), this type of change attests to the /49 high capacity of reflex regulation of the cardiovascular system as a whole.

Indexes of the functional tone of the blood vessels and cardiac activity were changed considerably during the time interval immediately preceding the moment of impact. Beginning 3-10 min before exposure, cardiac rate and arterial pressure began to increase as the moment of exposure approached. They gradually decreased 2-3 min later, approaching initial values. The character of these changes was identical in all experiments. The maximum increase in cardiac rate (up to 120-150 beats per min) usually took place 10-20 sec before the impact or during the first 10 sec thereafter. Meanwhile, the EKG showed a contraction for RR and RQ intervals and for the duration of the QRST ventricular complex in proportion to the increasing rhythm of myocardial contractions. The electric potential of the R and T waves in the second standard lead decreased in the majority of cases during the first 10 sec following exposure to impact acceleration shock (43 and 58.6 percent of the cases, respectively), while the voltage of the P wave increased (60 percent of the cases).

The relative decrease in the height of the R-wave was 0.15-0.2 mV; that of the T-wave, 0.12-0.14 mV. The P-wave increased 0.05-0.07 mV on the average. Analysis of the EKG recorded before exposure showed that the changes noted in the wave voltages were in the direction of normalization. Cardiac conduction was not disturbed by exposure to impact acceleration shock. Only one sub- /50 ject exhibited a slight increase in the incidence of a ventricular extrasystole. This, however, had been noticed prior to exposure and was due to individual intensification of autonomic excitability (ref. 25). Following exposure to 15.8 units in a "left side-right side" direction, see fig. 1, 6), the cardiac axis in one case shifted to the left and remained in that position for more than 2 min.

TABLE 2. CHANGE IN PULSE RATE OF SOME SUBJECTS AFTER A MEASURED PHYSICAL LOAD (MEAN DATA).

Subject	Change in pulse rate, beats per min					
	Before exposure			After exposure		
	At rest	Increase at end of first min	Increase at end of third min	At rest	Increase at end of first min	Increase at end of third min
M	76	+ 22	- 1.8	74	+ 22	- 0.7
D	68	+ 27	- 1.6	69	+ 24	- 5
D	72	+ 31	- 2	75	+ 32	+ 0.2
G	64	+ 29	- 1.2	66	+ 31	- 2.6
P	74	+ 41	0	76	+ 40	- 3.2
P	83	+ 30	+ 3	77	+ 34	+ 2
M	78	+ 30	+ 1.2	82	+ 33	- 0.8
P	80	+ 22	- 3	76	+ 22	- 4
V	66	+ 32	+ 0.6	79	+ 32	- 2.7
R	69	+ 2	- 2	68	+ 18	- 2

TABLE 3. CHANGE IN ARTERIAL PRESSURE OF SUBJECTS AFTER A MEASURED PHYSICAL LOAD (MEAN DATA).

Subject	Change in arterial pressure, mm Hg					
	Before exposure			After exposure		
	At rest	Increase at end of first min	Increase at end of third min	At rest	Increase at end of first min	Increase at end of third min
M	114/75	+ 11/- 0.8	- 0.4/- 0.4	116/76	+ 9/- 0.2	- 0.2/- 2
D	107/63	+ 14/- 0.4	0/- 0.4	103/67	+ 12/- 2	+ 1.4/- 2
D	113/74	+ 13/+ 1	+ 2.5/0	113/70	+ 17/- 3	+ 4.5/- 3.5
G	113/73	+ 9/- 3	+ 1/- 0.5	112/76	+ 12/0	+ 0.5/- 3
P	116/76	+ 20/+ 2	+ 7/- 1	113/77	+ 18/0	+ 2/0
P	119/78	+ 14/+ 5	+ 1.3/+ 1.3	119/84	+ 13/- 1.3	+ 1.3/- 1.3
M	129/86	+ 18/- 7	+ 2/- 2	126/90	+ 17/- 5	+ 3/- 4
P	125/80	+ 13/- 6	+ 3/- 5	121/75	+ 13/- 4	+ 1/- 3
V	123/67	+ 11/+ 0.7	- 4/+ 1.4	121/87	+ 13/+ 1.6	- 1.6/- 0.8
R	128/90	+ 13/- 2.5	+ 4/- 2.5	134/94	+ 6/- 6	- 1.3/- 1.3

This change was not accompanied by any subjective sensations. Comparable displacement of the heart was observed in experiments with monkeys (ref. 26). During the period of time (1-2 min) of the moment of exposure to impact acceleration shock, there occurred an increase in the cardiac beat and in the total amount of blood ejected by the ventricle.

This is borne out by the increase recorded in the duration and amplitude of the tones of the seismocardiogram. We should also point out the appearance, against the background of a rapid cardiac rate, of differences in the duration of the electric systole (calculated from the EKG data) and the mechanical systole (calculated from the data of the kinetocardiogram). Differences in the duration of the electric and mechanical systoles were sometimes as great as those observed diseases of the heart muscle (ref. 27). The short duration of this dysfunction (1-2 min) and its disappearance simultaneously with normalization of the pulse rate suggests that its cause lies in the temporary difficulty experienced /51 by the myocardium as a result of the large inflow of blood and rapidity of contractions. The picture of modified cardiac activity is accompanied by a concomitant though slower change in arterial pressure. The character of this change at different intervals in the preparation and performance of the experiment is shown in table 4.

Respiratory movements of the chest of the subjects likewise accelerated, reaching 24-30 per min, as the moment of exposure to acceleration shock approached. In some instances, the subjects deliberately held their breath at the moment of impact.

The character and magnitude of the changes noted in the cardiovascular system and respiration did not depend on the magnitude and direction of the impact acceleration shock (with the exception of a change in the cardiac axis). The external setting of the experiment and the emotional excitement of the subjects in anticipation of being subjected to an extreme factor largely influenced the character and degree of their reaction. Proof of this assumption may be found in the results of sham experiments, which duplicated the preparatory procedures and conduct of the experiment except for the exposure to acceleration. In these experiments the changes in pulse and respiratory rates and in the value of arterial pressure were even higher than in the regular experiments.

TABLE 4. DYNAMICS OF ARTERIAL PRESSURE--TIME INTERVAL IN RELATION TO THE MOMENT OF EXPOSURE TO ACCELERATION (MEAN VALUES FOR 112 CASES WITH THE ROOT-MEAN-SQUARE DEVIATION).

Arterial pressure	Value of arterial pressure, mm Hg				
	Before exposure			After exposure	
	20 min	2 min	1 min	1 min	2 min
Minimum	75(12)	75(12)	73(11)	70(12)	68(12)
Mean dynamic	98(15)	105(13)	106(11)	102(11)	100(11)
Lateral, piezometric	113(16)	126(17)	125(15)	122(15)	119(16)
Maximum	115(12)	125(8)	132(10)	128(13)	117(11)

The effect of emotional excitement was especially noticeable during the first experiments, when a heightening of the subjects' functions started 3-5 min before and was maintained throughout the entire preparatory period in the form of a generalized reaction. During the 4th or 5th experiment, this heightening was confined just to the moment of impact (fig. 2), which is a desirable reaction. /52

Observations on the preservation of purposeful movements by the subjects in response to a photic stimulation led to the conclusion that, following exposure to acceleration, a person can react rather quickly to external signals (0.5-1 sec).

Thus, our investigations show that a person can tolerate an impact acceleration of braking of considerable intensity without impairment of working ability or pathological changes.

The use of essential protective measures in the form of shock absorbing devices, safety belts, contour seats, etc. will make it possible to build aircraft capable of landing vertically at velocities of 8-10 m/sec.

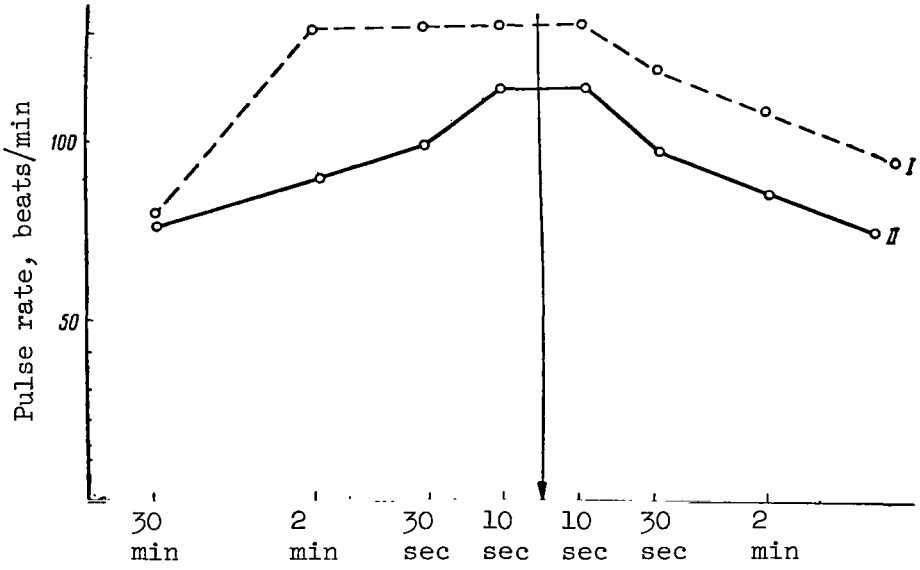

Figure 2. Dynamics of pulse rate in Subject V in first experiment (I) and in fourth experiment (II). Arrow indicates the moment of exposure to impact acceleration.

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THE PROBLEM OF FUNCTIONAL CHARACTERISTICS AND INTERACTION OF THE OTOLITHIC AND CUPULAR PORTIONS OF THE VESTIBULAR APPARATUS UNDER CONDITIONS OF ALTERED GRAVITY

Ye. M. Yuganov

It is impossible in the present communication to consider in detail /54 all the problems of vestibular physiology under conditions of altered gravity. Up to the present time, the study of applied problems in aviation medicine has had frequent recourse to the fundamental theoretical positions of the general physiology of sensors and the physiology of ear, nose and throat (ENT) organs, which were developed during the last century and are applicable to man while on the ground. In regard to mastery of spaceflight, investigators are faced with the problem of developing theoretical foundations for an "extraterrestrial" physiology of man in general and the physiology of his ENT organs in particular. There is no doubt that among the various reasons responsible for change in weight of persons in flight, weightlessness plays the greatest and more significant role in the inception of vestibular disturbances.

Research conducted in aircraft and spacecraft show that sensory and autonomic disorders of vestibular origin can develop in human beings after only brief exposure to weightlessness. The complex of vestibular-autonomic symptoms in practice bears a perfect resemblance to the clinical picture of motion sickness.

Examination of the problem of physiological mechanisms of space motion sickness indicates that its occurrence is connected with the disruption of the systemic operation of sensors, due to cessation of the action of gravity, to the influence of Coriolis accelerations occurring in displaced shifting of an individual and to increased excitation of receptor formations of the semicircular canals as a result of weakened inhibitory influences of the otolithic apparatus because of functional disconnection.

Functional disconnection of the otolithic apparatus and heightened sensitivity of the semicircular canals during weightlessness are consid- /55 ered to be the most probable hypothesis for explaining the mechanisms responsible for the onset of adverse vestibular disorders. It was proposed more than ten years ago and has been supported by a fairly large group of researchers (refs. 2, 3, 6, 10-13, 18 and 19). It is also widely believed that under such conditions a change occurs in the character and degree of stimulation of the otolithic apparatus, primarily in the form of diminished stimulation (refs. 2, 8, 9, 15 and 16).

Analysis of reports on the physiology of sensors shows that the problem of sensitivity of the vestibular apparatus during weightlessness has long attracted the lively interest of medical workers and biologists. However, experimental investigations of this area have been attempted for the first time only during the past 4-7 years. This period has seen a transition from the usual general theoretical discussions to broad painstaking research in laboratories on the ground and in space.

Attempts in this direction have consisted in determining the sensitivity of the otolithic apparatus under conditions simulating lowered weight. Experiments conducted in tanks have shown that subjects immersed in water acquire a rather marked raising of thresholds of sensitivity of the otolithic apparatus from changes in bodily position. Errors in determining bodily position average 22°, but may occasionally reach as much as 180° (refs. 7, 14, 16 and 20).

Unfortunately, all these experiments have been orientative, and they are consequently inadequate for determining the state of sensitivity of the otolithic apparatus during weightlessness. The fact is that tank experiments are only partially analogous to weightlessness; they are in essence studies of the sensitivity of the otolithic apparatus under conditions of partial deafferentation (exclusion of visual, partly tactile and temperature stimuli), while the otoliths themselves are actually not in a state of weightlessness. It is natural that this circumstance and certain other methodological difficulties point to the necessity for conducting investigations under outright conditions of weightlessness as reproduced in aircraft.

With the objective of solving this problem, the author's resources and attention were concentrated during 1961-1963 on studying the special features of the vestibular apparatus during altered gravity, especially in determining the condition of excitability of the vestibular apparatus during increased and lowered weight conditions.

We assumed that this would allow us to resolve one of the central theoretical and practical problems of space ENT physiology and to elucidate, first of all, whether weightlessness acts as an unusual stimulus to the otolithic apparatus by producing a specific stimulation of its neuroreceptor formations or whether weightlessness induces a functional disconnection of the otoliths as a kind of physiological labyrinthectomy.

If we found that weightlessness acts like any other stimulus, we /56
could expect that it would exert a cumulative action. It is natural that the summation of nervous processes taking place in this connection in corticosubcortical formations would subsequently explain the etiopathogenesis of space motion sickness.

In this way spaceflights introduced the task of studying completely new aspects of vestibular physiology. Moreover, these flights served as a unique laboratory for enriching general and applied physiology with new means of adequate external stimuli, thereby expanding the possibilities of physiological science itself.

Investigation of the special features of the functioning of the vestibular apparatus during altered gravity started with the study of the excitability of the otolithic apparatus during weightlessness, utilizing indirect and direct research methods.

We know that the study of the functional condition of the otolithic apparatus makes use of rather limited criteria and procedures. General physiological research saw widespread use of the determination of excitability of the otolithic apparatus during rocking and double rotation experiments. The advantages of these methods do not consist so much in recording certain special indexes, specifically reflecting the operation of the otolithic apparatus, as in applying direct external stimuli to this apparatus.

Knowing that the otolithic apparatus is functionally connected to the semicircular canal system, it would be justifiable to determine its state of excitation by simultaneous or successive application of stimuli to that system. To achieve this, it is sufficient to record changes in indexes characterizing the reaction of the semicircular canals to individual or combined action of stimuli adequate for one or another system.

This approach was used for indirect determination of excitability of the otolithic apparatus during decreased weight of the organism for direct investigation of its functioning. Direct methods included study of the excitability of the otolithic apparatus by application of galvanic current, determined by changes in threshold to the current during weightlessness.

Criteria for indirect evaluation of the functional condition of the otolithic apparatus included selected data which recorded the duration of postrotational nystagmus, the duration of illusion of counterrotation, the length of the latent period for the occurrence of the illusion of rocking and the threshold time of straightening out when tilting of the body took place.

The investigations showed that the duration of postrotational nystagmus during weightlessness decreased without exception in all 33 experiments. /57
It actually decreased 6-17 sec from the initial 19-30 sec. This pattern is convincingly confirmed in table 1, which presents comparative figures for the duration of nystagmus during weightlessness and on the ground for 10 subjects.

The strength of defensive movements during rotation was considerably less than on the ground. All subjects claimed that rotation during weightlessness was subjectively easier to undergo than on the ground. In a number of cases the illusion of counterrotation was not experienced. Its duration during weightlessness was noticeably shorter in the great majority of cases, as compared with the initial duration experienced on the ground. Pertinent figures are presented in table 2.

All data showed that the functional condition of the vestibular apparatus in man during weightlessness is characterized by a decrease in the duration of postrotational nystagmus, by shorter persistence of manifestation and /58
weaker expression of illusion of counterrotation and defensive movements during subjection of a person to angular accelerations.

TABLE 1. DURATION OF POSTROTATIONAL NYSTAGMUS OF SUBJECTS ON THE GROUND AND DURING WEIGHTLESSNESS (IN SEC).

Ground	Weightlessness	Δt, sec	Ground	Weightlessness	Δt, sec
24	18	-6	22	15	-7
26	16	-10	20	10	-10
21	14	-7	19	11	-8
20	13	-7	23	10	-13
26	13	-13	30	13	-17

TABLE 2. PERSISTENCE OF ILLUSION OF COUNTERROTATION IN SUBJECTS ON THE GROUND AND DURING WEIGHTLESSNESS (IN SEC).

Ground	Weightlessness	Δt, sec	Ground	Weightlessness	Δt, sec
12	10	-2	10	5	-5
12	9	-3	10	4.5	-5.5
10	7	-3	15	6	-10
10	6	-4	19	9	-10
5	0	-5	15.5	4	-11.5

Excitation of the vestibular apparatus by Coriolis accelerations during weightlessness was evaluated according to the duration of the latent period for the occurrence of the illusion of "rocking" and the size of the threshold time for subjects straightening out during the rotation period. The experiments showed that the latent period for the occurrence of illusion during weightlessness increases in relation to its size when taking place on the ground. Pertinent figures are presented in table 3.

The data in table 3 indicate that in the great majority of cases duration of the latent period of illusions in subjects is equal to 5.6-9.5 sec on the ground, but increases to 7.5-16.5 sec during weightlessness.

TABLE 3. DURATION OF LATENT PERIOD OF "ROCKING" ILLUSION ON THE GROUND AND DURING WEIGHTLESSNESS (IN SEC).

Ground	Weightlessness	Δt, sec	Ground	Weightlessness	Δt, sec
6.1	17.6	+11.5	5.6	18.0	+12.4
6.1	19.2	+13.1	5.6	20.8	+15.2
6.1	13.6	+7.5	5.6	20.9	+15.3
6.1	16.5	+10.4	6.6	18.5	+11.9
6.1	22.6	+16.5	6.6	21.6	+15.0

TABLE 4. THRESHOLD TIME FOR STRAIGHTENING OUT OF SUBJECTS AFTER ROTATION ON THE GROUND AND DURING WEIGHTLESSNESS (IN SEC).

Ground	Weightlessness	Δt, sec	Ground	Weightlessness	Δt, sec
3.0	2.5	-0.5	3.0	1.0	-2.0
3.0	2.0	-1.0	3.0	2.0	-1.0
3.0	1.5	-1.5	3.0	1.5	-1.5
5.5	2.4	-3.1	2.5	1.5	-1.0
3.0	1.5	-1.5	3.0	1.0	-2.0

In the second set of experiments, the subjects were subjected to Coriolis accelerations. Threshold time was determined for a subject to straighten out, after the torso had bent laterally. The figures for these experiments are presented in table 4.

Analysis of the data reveals that changes in the indexes of intensity /59 of human vestibular reactions to weightlessness are very regular. In practically all experiments and all subjects the latent period for the onset of reactions increased during weightlessness, but the intensity of the reactions decreased. This is confirmed by a decrease in the persistence of postrotational nystagmus, by decrease in duration of the illusion of counterrotation, by less marked sensations and defensive movements during angular acceleration, by an increase in the latent period for the onset of the "rocking" illusion

and by a decrease in the minimal period required for straightening out during Coriolis accelerations.

The data created an overall picture of lowered excitation for the semicircular part of the vestibular apparatus. Analysis indicates that this cannot be attributed to the specific effect of weightlessness on the cupular endolymphatic system, inasmuch as loss of weight cannot affect the inertial properties of the endolymph, and thus the character and degree of excitation of the semicircular canals.

The degree of excitation of the otolithic apparatus during weightlessness was elucidated by determining the threshold of this excitation to galvanic current.

Analysis of the data shows that during weightlessness a consistent change occurs in the threshold of excitation of the vestibular apparatus to such a stimulus. Not a single case occurred where the threshold value of the current, after producing a deviation in the degree of excitation of a subject's otolithic apparatus while on the ground, induced a similar reaction during weightlessness. It was necessary to increase the strength of the current by 0.5-2.7 mA in order to induce a deviation in the reaction of a subject during weightlessness.

In view of the brevity of weightlessness it was not always possible to determine the exact size of threshold excitation, even in 3-5 experiments. In a number of instances only the character of the threshold change could be determined. While this limited the value of the material, it produced valuable data for determining the character of threshold change and the special features of the functional condition of the sensor. Data contained in table 5 serve as illustrations of such changes in the experiments.

In order to eliminate completely the possibility of acceleration aftereffect on the threshold size of the vestibular sensor to galvanic current, 3 subjects executed 5 special flight experiments, during which weightlessness occurred immediately after a horizontal flight, without being preceded by the influence of acceleration. These cases produced qualitatively similar results. Pertinent data are presented in table 6.

Despite a comparable variation in the investigations, the possibility /60 of all these changes occurring as an acceleration postmanifestation could not be completely excluded. A wide range of investigations was therefore conducted regarding the excitation of the vestibular apparatus of a person under increased weight conditions. The investigations began by studying the excitation thresholds of the otolithic apparatus to galvanic current, the criterion being the onset of an illusion of banking. Pertinent data are presented in table 7.

The data obtained convinced us that the effects we had observed during weightlessness are also possible as acceleration postmanifestations. Moreover, the investigations were continued by utilizing indirect methods, especially by studying caloric nystagmic reaction to increased weight conditions. /61

TABLE 5. CHARACTER AND SIZE OF CHANGE OF EXCITATION THRESHOLDS OF THE VESTIBULAR APPARATUS TO GALVANIC CURRENT DURING WEIGHTLESSNESS (IN mA).

Ground	Weightlessness	mA	Ground	Weightlessness	mA
3.5	4.0	+0.5	3.0	3.5	+0.5
3.5	4.0	+0.5	3.5	4.9	+1.4
4.5	5.0	+0.5	2.5	5.2	+2.7
4.5	5.5	+1.0	2.5	4.0	+1.5
2.0	3.5	+1.5	4.0	5.5	+1.5
4.5	5.0	+0.5	3.5	4.9	+1.4
4.5	5.5	+1.0	2.5	5.2	+2.7
2.0	3.5	+1.5	2.5	4.0	+1.5
3.0	3.5	+0.5	4.0	5.5	+1.5

TABLE 6. CHARACTER AND SIZE OF EXCITATION THRESHOLDS OF THE VESTIBULAR APPARATUS TO GALVANIC CURRENT DURING WEIGHTLESSNESS (WITHOUT PRIOR ACCELERATION).

Condition of experiment	Threshold size of current, mA				
On the ground	2.0	2.0	3.5	3.5	2.5
During weightlessness	3.0	3.0	4.5	4.5	5.0

Data on the duration of nystagmus during acceleration of a constant magnitude are presented in table 8.

The caloric test was conducted 20 min before rotation and during the third minute of acceleration. Time discrepancy with respect to injecting water into an ear under these and other conditions was not more than 2-3 sec. Nystagmus was recorded with closed eyes. Electronystagmography was used. The biopotential leads utilized silver electrodes attached bitemporally to the outer edges of the eyelids; oscillograph tracing was recorded in ink. The latent period, duration and amplitude of caloric nystagmus were analyzed. Forty-two experiments were carried out on 20 subjects.

Comparative evaluation of the obtained experimental materials demonstrated convincingly that the duration of caloric nystagmus during acceleration in /62

TABLE 7. EXCITATION THRESHOLDS OF SUBJECTS' VESTIBULAR APPARATUS TO GALVANIC CURRENT DURING ACCELERATION.

	Threshold size of galvanic current, mA				
Ground	During acceleration				
	2 G	3 G	4 G	5 G	6 G
1.4	3.5	4.0	4.5	5.0	-
1.5	4.0	5.0	5.5	6.5	-
1.5	2.0	2.5	3.0	3.5	-
1.0	2.0	3.5	6.0	-	-
2.0	3.0	5.0	5.5	6.0	-
1.5	-	2.5	3.5	4.0	-
2.1	4.2	5.2	5.5	6.0	6.5
1.0	1.8	2.0	2.6	3.0	-
1.5	1.9	2.5	3.0	4.0	4.5
1.5	-	2.5	3.5	4.0	-

TABLE 8. DURATION OF CALORIC NYSTAGMUS OF SUBJECTS ON GROUND AND DURING 2 G ACCELERATION (IN SEC).

Ground	Acceleration (head-trunk)	Δt, sec	Ground	Acceleration (head-trunk)	Δt, sec
62	118	+56	0	105	+105
63	110	+47	0	140	+140
66	90	+36	0	133	+133
0	35	+35	115	184	+69
80	165	+85	17	88	+71

all cases increased considerably over the original value as recorded under ordinary conditions. As a rule, it increased 1.5-3 times.

TABLE 9. DURATION OF CALORIC NYSTAGMUS DURING MUSCULAR LOAD (IN SEC).

At rest	During load	Δt, sec	At rest	During load	Δt, sec
91	81	-10	125	118	-7
142	82	-50	135	104	-31
110	86	-24	113	52	-61
154	130	-24	84	57	-27
131	114	-17	87	58	-29

These isolated examples illustrate only the general direction of changes in nystagmic reaction during acceleration. Similar results were obtained in other experiments. In the great majority of cases, the latent period or second index of nystagmic reaction changed in characteristic fashion. It was found to be consistently shorter by 2-8 sec in duration as compared with the initial length. This could not be attributed to acceleration on neuroreceptor formations of the tacticle and motor sensors, because control experiments (table 9) demonstrated that their stimulation inhibits nystagmic reaction.

The experiments showed qualitatively contrasting changes in the duration of the nystagmic reaction to acceleration and weightlessness. The hypothesis that weightlessness has the same effect as physiological labyrinthectomy inevitably assumes the absence of effects during weightlessness, stemming from the functional interaction of the otolithic and cupular apparatuses. According to Voyachek-Khilov's theory, it aims at increasing the excitability of the cupular apparatus, a frequent manifestation of which should be the triggering of vestibular nystagmic persistence. As our experiments have shown, reversible physiological phenomena occur during weightlessness, but triggering of the nystagmic reaction is characteristic of acceleration.

Analysis of the data shows that weightlessness does not produce a functional disconnection of the otolithic apparatus, i.e., a physiological /63 labyrinthectomy, but acts rather as an unusual minus-stimulus responsible for the onset of specific vestibular reaction.

The experimental data proved interesting for other reasons as well. They are responsible for the conviction that acceleration, i.e., centrifugal forces, induces the nystagmic reaction.

This thesis completely contradicts the generally accepted view of classical physiology regarding the vestibular apparatus. We know that as far back as the First All-Union Congress of Laryngologists in Leningrad (1924), V. I. Voyachek, in explaining the mechanism of brief rotational nystagmus, proposed the hypothesis that irritations of the otolithic apparatus caused by the action of

TABLE 10. DURATION OF CALORIC NYSTAGMUS DURING ACCELERATION (2 G) IN CHEST-BACK POSITION, AND INCREASE IN NYSTAGMIC DURATION DURING 3-4 G ACCELERATION.

Duration of nystagmus at 2 G	Δt of nystagmus (sec) during acceleration		Duration of nystagmus at 2 G	Δt of nystagmus (sec) during acceleration	
	3 G	4 G		3 G	4 G
115	+11	-	153	+24	-
144	+30	-	94	+22	+10
118	+32	-	116	+35	-
93	+16	+7	89	+34	-
110	+26	-	118	+23	-

centrifugal forces would exert an inhibiting influence on the operation of the semicircular canals. This was subsequently definitively confirmed by the experimental work of K. L. Khilov (refs. 5 and 6).

In view of the manifest contradiction between our experimental materials and K. L. Khilov's theory, a supplementary series of experiments was conducted for the purpose of refining the results of our research. It was assumed that if centrifugal forces of constant magnitude actually activate the nystagmic reaction, then on cessation of caloric nystagmus to a 2 G acceleration, its subsequent increase to 3 G after 10-20 sec and then to 4 G should again bring on nystagmus for each of these conditions. The experiments definitively confirm our supposition. Table 10 gives the size of increase for caloric nystagmus in the subjects after application of 3 G acceleration relative to nystagmus from 2 G acceleration and of 4 G relative to 3 G acceleration.

The findings show that caloric nystagmus recurred in the subjects /64 after 3 G acceleration. It was 11-35 sec longer than that with 2 G acceleration. If, after cessation of caloric nystagmus from 3 G acceleration, 4 G acceleration was applied, an "activated" nystagmus of 7-10 sec duration followed.

The comparative size of increase in the duration of nystagmus under altered gravity points to a gradual decrease in Δt of the nystagmus in proportion to the increase in the magnitude of acceleration. We received the impression that acceleration, constant in magnitude and direction, during the period of stimulating the semicircular canals would definitely activate the nystagmic reaction. On the other hand, any change in magnitude or direction would exert an inhibiting influence on the nystagmic reaction. Two special series of investigations were conducted for the purpose of rendering this supposition more precisely.

TABLE 11. DURATION OF CALORIC NYSTAGMUS DURING STEADY (150 SEC) INCREASE AND DECREASE IN ACCELERATION.

Magnitude				Magnitude			
1 G	2 G	1↗2 G	2↘1 G	1 G	2 G	1↗2 G	2↘1 G
121	137	159 (1.8)	110 (1.2)	83	94	101 (1.7)	80 (1.1)
94	115	122 (1.8)	66 (1.6)	178	213	220 (2.0)	128 (1.3)
85	147	176 (1.7)	82 (1.4)	58	91	149 (1.5)	44 (1.8)
135	149	152 (1.8)	122 (1.2)	57	66	69 (1.4)	22 (1.9)
84	104	112 (1.8)	82 (1.4)	60	70	130 (1.9)	68 (1.7)

Special procedural features established the relationship between the condition of the nystagmic reaction and both the value of the absolute magnitude of the acceleration and the actual process of magnitude modification. While adhering to these conditions, 34 experiments were carried out on 20 subjects. The data for 10 subjects of these experiments are presented in table 11.

The data in the table point out two important manifestations. First, they indicate the undoubted increase in the duration of caloric nystagmus in experiments with a gradual increase in the magnitude of acceleration from 1-2 G, relative to the persistence of nystagmus obtained in experiments with a constant 2 G acceleration.

This also applies to shorter persistence of caloric nystagmus in /65 experiments with a constant decrease in magnitude of acceleration from 2-1 G as compared to the duration of nystagmus from constant application of a 1 G acceleration. "Increasing acceleration" profile experiments show that duration of nystagmus is 3-58 sec longer than the duration for 2 G. "Decreasing acceleration" profile experiments show the nystagmus to be 3-50 sec shorter than for the 1 G tests.

The second manifestation can serve as a typical example of the results obtained in all other experiments. The duration of caloric nystagmus in a subject (ref. 8) was equal to 91 sec under 2 G acceleration. It was just as long for him in an increasing acceleration profile experiment, except that it began after 1.5 G rather than 2 G acceleration. In the succeeding stepwise increase of acceleration to 2 G, the duration of nystagmus reached 149 sec.

It must therefore be acknowledged that it is not only the absolute magnitude of acceleration, but the process of modifying its magnitude and the type of change which determine the strength and character of nystagmic reaction in man.

The experimental data allow a direct answer concerning the special features of some reactions of the semicircular canals under conditions of increased weight in man, and the dependence of such reactions on the functional condition of the otolithic apparatus during constant and increasing acceleration. These data confirm the general thesis of Voyachek and Khilov concerning a connection between the otolithic apparatus and the semicircular canals. Furthermore, they expand our ideas of the special features of this interconnection.

It must be assumed that K. L. Khilov's concept of the inhibiting influence of otolithic stimuli, particularly of centrifugal force, on the strength of nystagmic reaction is more limited in significance than has been heretofore acknowledged. Our data do not negate this concept; they only delimit more strictly the sphere and place of its application in a detailed analysis of experimental phenomena at the moment of simultaneous or consecutive application of stimuli to the cupular endolymphatic and otolithic apparatus.

K. L. Khilov's conclusion concerning the inhibition of the vestibular nystagmic reaction following the application of a centrifugal force to an organism is probably not as broad in scope, inasmuch as it refers to a particular instance of such application. Evidently the character and strength of nystagmus following the application of centrifugal force can be correctly evaluated only if we take into account the character of the force applied /66 (constant or variable), its absolute strength and the character of change of this force.

The detail and variability of our experiments elucidated certain important and new aspects of the interaction of the otolithic apparatus and the semicircular canals. We believe that inhibition of vestibular nystagmus by application of otolithic stimuli does not exhaust all special features of the functional interconnection between the semicircular canals and the otolithic apparatus.

The character of this interaction will vary, depending on the quality of stimuli externally applied to the otolithic apparatus. Stimulation of the otolithic apparatus can produce both inhibition and activation of the nystagmic reaction. This should be considered as the external manifestation of qualitatively different forms of the interaction of the semicircular canals and the otolithic apparatus.

Our experiments demonstrate that any acceleration of constant magnitude, exceeding the ordinary weight of the organism, intensifies the strength of the fundamental indexes of vestibular nystagmic reaction in the same way as do increases in the magnitude of acceleration. Moreover, decrease in this magnitude by an external agent leads to inhibition of the nystagmic reaction.

The findings of earlier authors, relating nystagmic inhibition solely to post- and prior-angular acceleration of the application of centrifugal force to the otolithic apparatus, should probably be evaluated from this point of view. We are convinced that the physiological effects, by these authors, were not due to temporary special features in the application of centrifugal force relative to the moment of operation of angular acceleration, nor were they

due to some special properties of centrifugal force having an inhibiting effect. They are rather due to the selective characteristics of centrifugal forces used by the authors in their experiments.

The fact is that T. D. Bakhvalova, rotating animals on the periphery of a centrifuge with constant acceleration, observed a shortening of nystagmus as compared with rotating them at the center of the apparatus. Bakhvalova attributed this phenomenon to the action of centrifugal force preceding the effect of angular accelerations. It must be admitted that this fallacious presentation is in need of rectification.

In T. D. Bakhvalova's experiments the animals were subjected to constant acceleration, up to the moment of braking the centrifuge; this produced a nystagmic reaction. The period of braking the centrifuge was used to exert negative angular accelerations and a simultaneous decrease in the magnitude of /67 acceleration from 1.2-1 G. The decrease in magnitude of acceleration was not considered a possible reason for changing the duration of nystagmus; nor was it used by the author in evaluating the results of the experiment. Yet this decrease, rather than the preceding action of acceleration, was the responsible cause for inhibiting nystagmic reaction.

The same considerations should be used in evaluating the experiments of A. P. Popov, which were carried out on human beings by a similar method. The author's assertions concerning shortening of nystagmic duration in direct proportion to the size of the radius of rotation, i.e., to the size of the centrifugal force, is also unacceptable.

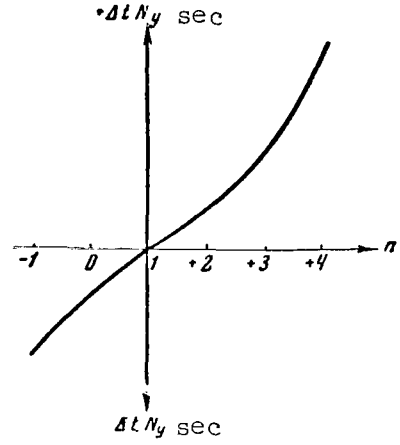
It is natural that any shifting of the animal towards the periphery would be accompanied by an increase in the operating acceleration. However, this fact does not mean by any means that the degree of inhibition of the nystagmic reaction is determined by the magnitude of a constantly acting acceleration. We have already pointed out that accelerations with such a characteristic are responsible for the duration of nystagmus.

The strength of nystagmic inhibition in this case depends on the degree of change in the magnitude of the acceleration, inasmuch as the inhibiting effect of nystagmic reaction becomes all the more significant as this degree increases in value. For this reason it makes a difference whether the change in acceleration is from 2-1 G or from 1.2-1 G. It is such factors in regard to external forces that the authors encountered in their experiments. Unfortunately, these externally perceptible factors (dissimilar magnitudes of preceding accelerations) were assumed by them to be genuinely responsible for the varying degree of nystagmic inhibition. Actually they were the result of the organism being acted on by decreasing acceleration of varying magnitude.

Our experimental data permitted us to make a different evaluation of the findings of earlier experiments. They also enabled us to describe the effect of operation of acceleration on the otolithic apparatus in man. The experimental data show that each time constant or increasing acceleration is applied in the direction of head-trunk to a man, thus increasing his weight, a nystagmic reaction is activated.

TABLE 12. DURATION OF CALORIC NYSTAGMUS UNDER 1 AND 2 G ACCELERATION ON THE GROUND.

+ 2 G	Ground	-1 G	+2 G	Ground	-1 G
124	100	25	135	102	87
116	99	85	100	67	30
118	86	60	94	26	0
69	27	0	178	155	27
118	87	60	60	15	0



This is always accompanied by an increase in the pressure of otolithic formations on the neuroreceptor apparatus of the utricular part of the vestibular apparatus relative to the size of pressure of the otoliths on similar formations in man under ordinary ground conditions. Moreover, each time that a smaller acceleration acts on the organism, thus decreasing its weight, /68 inhibition of nystagmic reaction occurs in the individual. Such an action has been accompanied by a decrease in pressure of otoliths on the receptor formations of the utricular apparatus.

For the purpose of confirming this assertion, a series of experiments was conducted with acceleration of 1 G; the findings are presented in table 12.

We found that the application of a 1 G negative acceleration considerably decreases the duration and amplitude of caloric nystagmus in all cases and for all subjects (see illustration above).

Our findings regarding changes in nystagmic reaction under conditions of increased and zero weight and during the application of negative acceleration reflect the qualitatively varied character of excitation of the otolithic

apparatus under these conditions and uniqueness in the functioning and interaction of the otolithic and cupular apparatus during increased and decreased weight in man. /69

The ideas presented should not be considered definitive. The data show that the problem has been resolved primarily on the basis of data characterizing changes in certain indexes of vestibular motor reactions. Once the character and degree of change for the indexes of vestibular vegetative reflexes have been studied, it would be natural for subsequent investigations to expand and refine the data relating to the special features in the functioning and interaction of different divisions of the vestibular apparatus under conditions of weight modification.

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THE PROBLEM OF HUMAN RESISTANCE TO SHORT-TERM ANGULAR ACCELERATIONS OF LARGE MAGNITUDES

V. M. Tardov, B. V. Ustyushin and S. F. Orlov

At the present time, in connection with the problem of spaceflights, /70 the study of the effects of various accelerations on man has acquired particularly great importance.

The present work is devoted to investigation of the effect of angular accelerations of short duration and large magnitudes (±30-±90 units/sec^2) on man subjected to rotation about the longitudinal axis of the body, and represents a new departure in such investigations, there being no literature on the subject.

The test subjects were 6 healthy males aged 22 to 25 years.

The necessary angular accelerations were created on a special test stand (designed by V. V. Dobrynin) consisting of a chair, power clutches and a damping system, mechanisms for starting and automatic braking, and a device for recording rotation time, angles of rotation, and tangential acceleration. The clutches and damping system insured that the moment of torque would be imparted to the chair in the course of 0.2 sec. The automatic braking system insured that stopping the chair also took 0.2 sec. Thus, the effect of clockwise (positive) angular acceleration lasted 0.2 sec, and was replaced by a deceleration (negative angular acceleration) lasting the same length of time. In addition, several experiments were conducted in which the braking system was not activated, and after cessation of positive angular acceleration the chair and test subject continued to rotate by inertia, gradually coming to a stop by the force of friction.

The investigations were begun at 30 units/sec^2. From one experiment to the next the magnitude of the active factor grew by 10 units to 90/sec^2. /71 Altogether 56 experiments were conducted.

The factors studied were the general state of the test subject, the reaction of his vestibular apparatus and the cardiovascular, respiratory and nervous systems.

The following physiological indices were recorded: electronystagmogram, EKG in three standard leads, arterial pressure, respiration rate and EEG in two

variants (bipolar fronto-occipital and unipolar occipital). Functional records were made on an electroencephalograph and multichannel pen-writing recorder.

Analysis of subjective reactions and objective data showed that all subjects withstood well the ±30-±90 units/sec^2 range, the magnitude not significantly affecting the results. Short-duration, large-magnitude accelerations failed to produce a single case of nystagmus, a fact of special interest, while milder, but more prolonged accelerations (0.3-0.5 units/sec^2, 17-32 sec) produced nystagmus in every instance (figs. 1 and 2). It must be noted that subjective sensations during short-duration effects of angular acceleration were characteristic for adequate stimulation of the semicircular canals. The subjects felt these effects in the form of a sensation of turning and listing, first in one direction and then in the reverse. From this circumstance /72 and the absence of nystagmus we concluded that although large, brief angular accelerations (to 0.2 sec) are an adequate stimulant for the labyrinth, they are a relatively weak one.

Definite changes were observed in the subject's cardiovascular and respiratory systems. The reaction of the former expressed itself chiefly in a higher rate of cardiac contraction immediately after the chair stopped. In separate instances the frequency of cardiac contractions at that moment reached 125/min, after which the pulse rate as a rule quickly dropped, reaching the initial level within 5-15 min and even falling below it (fig. 3).

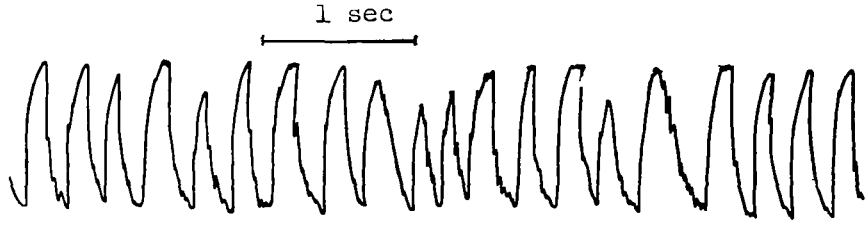

Figure 1. Electronystagmogram in experiment with negative angular acceleration of 0.4 units/sec^2 lasting 31 sec (subject No. 2).

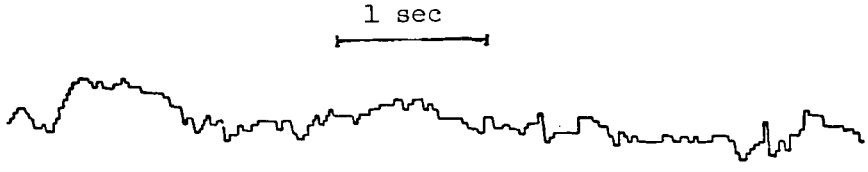

Figure 2. Electronystagmogram in experiment with angular acceleration +80 units/sec^2 (subject No. 2). Nystagmus is absent.

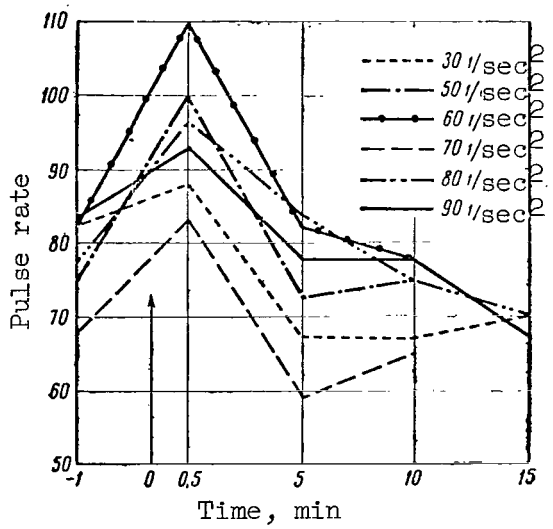

Figure 3. Change in frequency of cardiac contractions during short-term angular accelerations of ± 30 to ± 90 units/sec^2 (subject U).

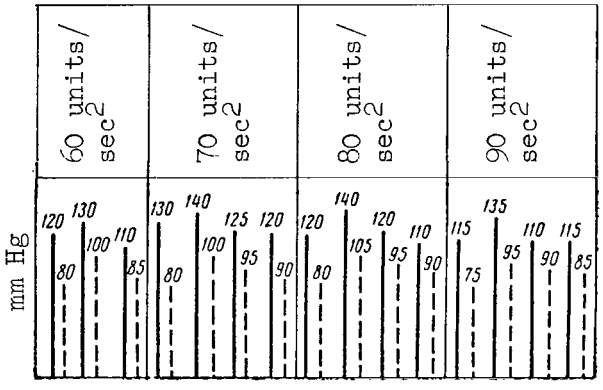

Figure 4. Change in arterial pressure during short-term angular accelerations of ± 60 to ± 90 units/sec^2 (subject D).

Analogous changes were observed in blood pressure. Immediately upon cessation of angular acceleration both systolic and diastolic pressure increased, with pulse pressure hardly changing (fig. 4). Some 20-30 min later systolic pressure returned to the initial level, while the diastolic pressure, although declining, remained above this level, which led to a certain reduction in pulse pressure.

Analysis of EKG curves showed a definite parallelism between the dynamics of the rate of cardiac contraction and the EKG elements. Thus, the QT interval (electric systole of the heart) quickened with an increase in the rate of cardiac

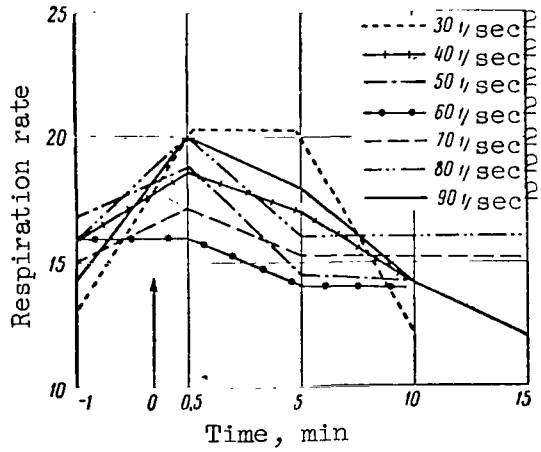

Figure 5. Change in respiration rate during short-term angular accelerations of ± 30 to ± 90 units/sec^2 (subject U).

contraction, while 10-15 min later it returned to the initial level. The QT changes were somewhat shortened with respect to changes in the frequency of cardiac contractions. The systolic index (according to the Fogel'son-Chernogorov method) in the period of greatest rate of cardiac contraction rose by 10-15 percent compared to the initial level, and then rapidly returned to normal. The spike voltage underwent no substantial changes in all experiments.

Changes in the respiratory function were manifested by increase in /74 respiration rate immediately after short-duration angular acceleration. The rate quickly returned to normal levels (fig. 5).

An analysis of EEGs showed an increase in the amplitude and content of high-frequency rhythms (greater than 20 cps) immediately after acceleration, which indicates the predominance at that moment of excitatory processes in the central nervous system.

Assessing all the data cited above, we note that the responsive reaction of the organism to short-term angular acceleration of large magnitudes is in essence not specific for this active factor. We should rather speak of a responsive reaction to the effects of mechanical energy and of a neuropsychiatric reaction as a result of emotional stress, such as occurs in catapulting. This proposition was confirmed by control experiments, in which the subject, awaiting rotation and hearing the command to set the chair in motion, is then not subjected to rotation. In this case all those changes in physiological functions are observed which appear during true rotation, but they are less pronounced.

CHANGES IN SEVERAL PHYSIOLOGICAL AND BIOCHEMICAL INDICES IN MAN AFTER EXPOSURE TO SMALL CONCENTRATIONS OF CARBON MONOXIDE

V. V. Kustov, V. I. Mikhaylov, Z. I. Pilipyuk, Yu. N. Tokarev, V. S. Georgiyevskiy, B. S. Katkovskiy and A. N. Kalinina

The air in hermetically sealed rooms in which man is present /75 invariably contains carbon monoxide, a product of his vital activity. The release of carbon monoxide into the environment with the air exhaled by man was first noted by Jackson (ref. 10). Subsequently, large quantities of endogenous carbon monoxide were detected in the air exhaled by man (refs. 3, 6 and 9). We now present some data on the carbon monoxide content of the air exhaled by man (mg/liter):

Author	mg/liter
Jackson	Traces
Sjöstrand	0.0028
Bogatkov et al.	0.10-0.015
Kustov	0.011

Since these concentrations are close to the maximum permissible in the air of industrial establishments, we thought it would be interesting to study the effect of CO in a concentration of 0.011 mg/liter (which, according to our findings, corresponds to its content in the air exhaled by man) on some biochemical and physiological indices in man.

Healthy nonsmoking males 21-24 years of age were the subjects in our experiments. /77

Each subject took part in an 8-hour background experiment (hermetic sealing) and in an 8-hour experiment involving exposure to carbon monoxide.

The CO concentration of the chamber (0.011-0.012 mg/liter) was maintained with the help of an automatic measuring device (ref. 5). The CO_2 content did not exceed 0.6 percent, while the air temperature was 18-22°C and the relative humidity 50-60 percent.

All subjects were tested before and after the experiment for blood catalase activity by the method described in reference 2, serum cholinesterase activity by the method described in reference 7 and blood carboxyhemoglobin content in a Van Slyke apparatus.

TABLE 1. EFFECTS OF 8 HOURS OF HERMETIC SEALING AND EXPOSURE TO CONCENTRATION OF 0.011-0.012 mg/liter OF CARBON MONOXIDE ON SOME INDICES OF EXTERNAL RESPIRATION (MEAN DATA)

Indices	OUF $\frac{\text{mg } O_2}{\text{1 liter of air}}$				O_2 consumption in 1 min at rest			
	before	after	Change in external respiration, %	P	before	after	Change in O_2 consumption, %	P
Hermetic sealing M ± m	42.8 ± 1.4	46.4 ± 1.6	108.4	> 0.05	263.3 ± 7.3	280.3 ± 7.3	107.6	> 0.05
No. of observations	8	7			8	9		
Effect of carbon monoxide M ± m	41.8 ± 1.7	30.8 ± 1.4	73.68	> 0.01	264.2 ± 11.4	231.0 ± 15.9	987.5	> 0.05
No. of observations	8	10			7	7		

Table 1 (Continued)

Indices	MRV, liters				Respiratory rate			
	before	after	Change in MRV, %	P	before	after	Change in respiratory rate, %	P
Hermetic sealing M ± m	6.03 ± 0.39	6.38 ± 0.30	105.8	> 0.05	13.4 ± 0.56	12.7 ± 0.39	94.8	> 0.05
No. of observations	7	8			7	7		
Effect of carbon monoxide M ± m	6.50 ± 0.49	6.47 ± 0.29	99.5	> 0.05	12.7 ± 0.4	13.0 ± 0.4	102.3	> 0.05
No. of observations	9	7			7	7		

Note. OUF - oxygen utilization factor, MRV - minute respiratory volume.

Before and after the experiment the following were recorded: EKG in the three standard leads, level of arterial pressure, oxygen consumption and utilization factor (amount of O_2 consumed from 1 liter of air passing through the lungs). Some psychological tests were also taken (working with number and letter tables and arithmetical counting with switching).

The subjects were under observation throughout the experiment (note was taken of their complaints; the pulse and respiratory rate were recorded). EKGs were recorded the second and sixth hours of observation.

The experiments showed that 7-8 hours after the start of the experiment the subjects exhibited some functional changes in the cardiovascular system and external respiratory function, as well as slightly decreased efficiency /78 in performing the psychological tests.

For example, analysis of the EKGs recorded on a 4-PFD-7 showed that in 7 out of 10 subjects, voltage of the P, R and T waves decreased (generally in the second standard lead) as did the total voltage of the waves, while there was some widening of the QRS complex (associated in some cases with acceleration of the cardiac rate).

Table 1 shows the changes in some indices of external respiration noted in the background experiments and in the experiments involving exposure to carbon monoxide.

It is evident from table 2 that after 8 hours of exposure to carbon monoxide in a concentration of 0.011-0.012 mg/liter all subjects made more mistakes in the psychological test (counting with switching) than they did in the experiment with hermetic sealing. Since the same result occurred in every case (increased number of mistakes), the reliability of the difference between the experiment and the control, calculated by means of the criterion of "signs" (refs. 1 and 4), amounted to 99 percent, i.e., it was significant. However, the efficiency in handling the arithmetic tables did not change /79 ($P > 0.01$).

The data on the changes in some biochemical indices are summarized in table 3.

It is evident from table 3 that after exposure to carbon monoxide the blood carboxyhemoglobin content rose from 0.66 ± 0.056 to 1.58 ± 0.43 percent. At the same time there was a statistically significant increase in serum cholinesterase activity. Blood catalase activity did not change ($P > 0.05$).

Analysis of the data suggests that the minimum physiological shifts observed in the subjects after exposure to CO were not due solely to carbon monoxide hypoxemia, because the blood carboxyhemoglobin content was only 1.58 percent. We are therefore inclined to ascribe these changes to the tissue effect of carbon monoxide and to take them into account in determining the average daily maximum permissible concentration of CO in the air of airtight places.

TABLE 2. EFFECTS OF 8 HOURS OF EXPOSURE TO CARBON MONOXIDE ON SUBJECTS' PSYCHOLOGICAL ACTIVITY.

Subject	Background experiment (without CO_2)		Effect of CO_2	
	Test 2 (arithmetic counting)			
	No. of mistakes in 10 min of work, %	Efficiency in 10 min of work (arithmetic mean)	No. of mistakes in 10 min of work, %	Efficiency in 10 min of work (arithmetic mean)
K.	4.0	12.6	19.0	22.1
K.	3.0	10.7	6.0	13.7
Kh.	0.0	17.3	1.4	14.1
Sh.	0.0	18.6	0.5	19.1
S.	0.0	19.9	1.8	22.8
A.	3.0	12.4	4.0	14.8
P.	6.0	8.9	14.0	11.7
Kh.	0.0	17.3	0.4	22.6
Mean	2 ± 0.75	14.96 ± 1.37	5.87 ± 2.3	17.62 ± 1.37

TABLE 3. EFFECTS OF 8 HOURS OF HERMETIC SEALING AND EXPOSURE TO CARBON MONOXIDE IN CONCENTRATION OF 0.011-0.012 mg/liter ON SOME BIOCHEMICAL INDICES IN SUBJECTS.

Experimental conditions	Carboxyhemoglobin content, % (M ± m)			Serum cholinesterase activity, % (M ± m)			Catalase index (M ± m)		
	Before	After	P	Before	After	P	Before	After	P
Hermetic sealing	1.48 ± 0.48	0.516 ± 0.26	< 0.001	100	92.1 ± 3.1	< 0.05	3.62 ± 0.17	4.42 ± 0.16	< 0.002
Number of observations	6	6		6	6		10	10	
Exposure to carbon monoxide	0.66 ± 0.56	1.58 ± 0.43	< 0.002	100	107 ± 2.4	< 0.05	3.70 ± 0.74	3.73 ± 0.25	< 0.05
Number of observations	7	7		6	6		6	6	

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EFFECT OF ACCLIMATIZATION TO HIGH-MOUNTAIN CONDITIONS ON HUMAN RESISTANCE TO HYPOXIA

Ye. N. Salatsinskaya

It is a well-known fact that physical training and acclimatization to /80
hypoxia increase resistance to stress. The physiological mechanisms of athletic training and acclimatization to hypoxia are similar. In both cases the body experiences oxygen starvation, which results in the alteration of several physiological functions (ref. 2).

Athletic training increases the number of erythrocytes, hemoglobin and glutathione concentrations of the blood, etc. It improves physical and moral-volitional qualities and strengthens the functional capabilities of the analyzers and the ability of the body to adapt to changing environmental conditions. Training is a means of combatting fatigue and improving physical fitness. Athletes are more capable than others of tolerating a high-altitude climate (refs. 3, 5 and 6).

Hematopoiesis is stimulated in the course of acclimatization. The tissue respiratory enzymes are altered, resulting in decreased sensitivity to ionizing radiation (refs. 2 and 8).

Thus, the demands made on the cosmonaut are similar to those made on the mountain climber (acclimatization, physical and vestibular conditioning, swift reaction to threatening conditions, daring, capacity for working under unusual conditions, etc.).

There are references in the foreign literature to the fact that in selecting cosmonauts we should be guided by the data obtained from examination of the inhabitants of mountainous regions 4,000 m or more above sea level (ref. 9). They have developed high tissue resistance in the course of prolonged /81
acclimatization (over several generations). As a result, their bodies are capable of withstanding low oxygen pressure without suffering central nervous disturbances. "Mountain Indians have unusually effective muscular tissue" (ref. 2).

In view of the foregoing, it seemed important to determine the difference between acclimatized and nonacclimatized individuals in their reactions to physical exertion at high altitudes to hypoxia, and to study the effect of fatigue during ascents.

We therefore undertook to investigate 38 mountain climbers in the Caucasus at an altitude of 1,650 m above sea level. All subjects had received pre-camp

physical training (before leaving for the mountains). Twenty-one of them had previously gone on marches and climbed mountains, i.e., they were acclimatized. The other subjects were not acclimatized. Both groups remained in the camp 20 days, during which they followed a conditioning program, went on marches and climbed to an altitude of 4,100 m above sea level. Consequently, by the end of the stay in camp, the second group had about as much acclimatization as the first group before the start of the investigation. Both groups were examined when they first came to camp, before and after each climb, and at the end of the stay in camp.

The investigations involved a determination of resistance to hypoxia from the duration of respiration in a Krogh apparatus filled with air. As oxygen was consumed, the amount decreased and the carbon dioxide was absorbed by soda lime. The amplitude and rate of respiration were recorded on a kymograph at the same time. The subject's handwriting was checked while he was describing how he felt. These investigations were paralleled by a recording of oxygen saturation of the blood on an oxyhemograph.

The experiment ended with the onset of marked cyanosis or tremor. These phenomena did not appear in several subjects, in which cases a change in handwriting was a signal to halt the investigation.

This method was used in a total of 204 investigations and 160 determinations of the hemoglobin concentration of the blood. In order to ensure identical conditions, the investigations were conducted early in the morning, immediately after arising, before exercises and morning toilet.

Since we attached considerable significance to the combined effect of physical exertion and hypoxia on physically trained people, we determined resistance to hypoxia just before an ascent and immediately after the return to camp.

The results of these investigations revealed insignificant abnormalities among the acclimatized persons in resistance to hypoxia in either direction./82 Most of the subjects who completed two ascents reacted differently to hypoxia. The mean data for this group were naturally statistically insignificant, showing a slight increase (3.1 percent). The nonacclimatized subjects, on the other hand, exhibited a statistically significant decrease in resistance, averaging 12.3 percent. Consequently, physical exertion in the form of climbing to great heights produced a more unfavorable reaction to hypoxia in the nonacclimatized subjects than it did in the acclimatized. This shows that acclimatization has a beneficial effect on physical exertion.

Resistance to hypoxia after 20 days in the mountains increased in both the acclimatized and the nonacclimatized subjects by 19.9 and 20.4 percent, respectively.

During the determination of resistance to hypoxia, the level of pulmonary ventilation rose in both groups almost equally, in a Krogh apparatus, until the 7th minute of respiration. Ventilation then continued to increase in the acclimatized, while it dropped in the nonacclimatized. These changes resulted from a decrease in the amplitude of respiration with insignificant changes in

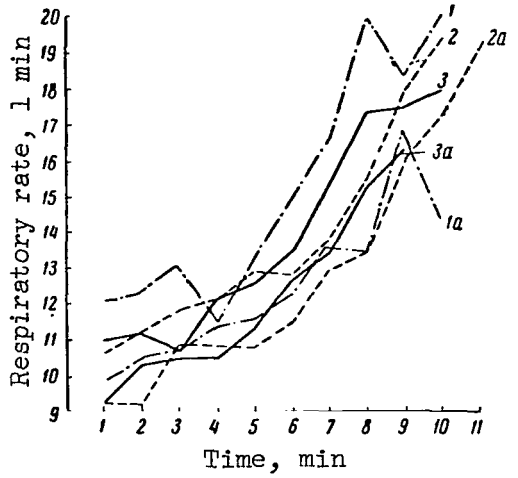

Figure 1. Change in respiratory rate in a hypoxic test during the stay in the mountains. 1, the first day in camp; 2, before an ascent; 3, after the ascent. The figures with letter a, acclimatized persons; without letter a, nonacclimatized persons.

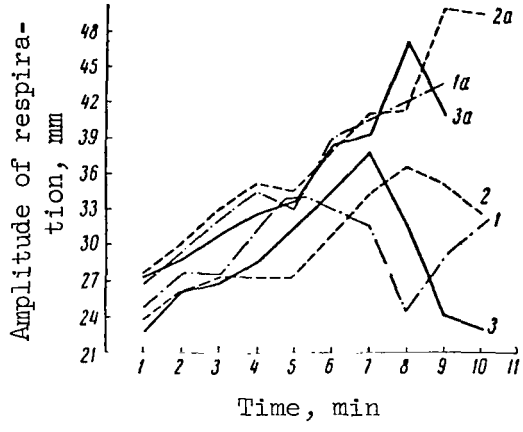

Figure 2. Change in amplitude of respiration while in camp and during an ascent. Symbols are the same as in figure 1.

the respiratory rate (figs. 1 and 2). The increase in volume of pulmonary ventilation in our subjects was evidently due to marked fatigue of the respiratory muscles in those not acclimatized to heights. The main role here was probably played by the conditioning of respiratory excursions in the acclimatized, which arose as a result of the effect of low partial pressure of oxygen and wearing the knapsack.

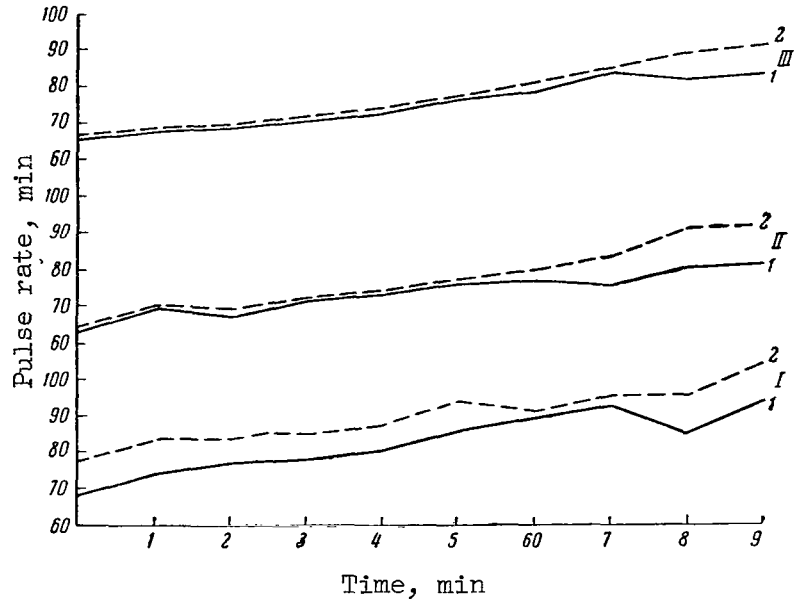

Figure 3. Dynamics of cardiac rate during the determination of resistance to hypoxia. I, first examination (first 24 hours in camp); II, examination before an ascent; III, examination after the ascent; 1, acclimatized; 2, nonacclimatized.

Baychenko, Budanov and Krestovnikov found (ref. 1) that altitude has a rather marked effect on nonacclimatized persons and reduces the efficiency of their respiratory muscles.

During the first determination of hypoxic resistance (first 24 hours in camp), the cardiac rate in the nonacclimatized subjects was faster than in the acclimatized (fig. 3). During the second determination (after 6-15 days in camp), the difference in cardiac rate leveled out to a great extent, as it dropped more in the nonacclimatized than it did in the acclimatized.

The change in oxygen saturation of the blood, determined by oxyhemography during the test for hypoxia, showed that oxygen saturation of the blood prior to the ascent was less in the acclimatized subjects than in the nonacclimatized, a sign of greater adaptability to hypoxia in the acclimatized. During an ascent the rate of saturation in both groups dropped almost equally, apparently because both groups continued to adapt to altitude (fig. 4). For /84 convenience of comparison, the degree of reduction of oxygen saturation was determined from the following formula that we devised

$$A_p = \frac{96 - R_{oxy}}{T_p},$$

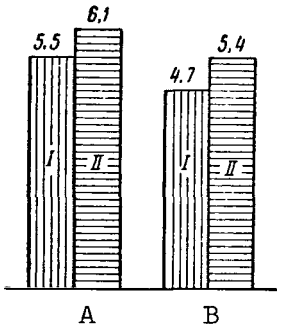

Figure 4. Rate of drop of oxygen saturation of the blood (in seconds) during the hypoxic test (mean data from the groups in percent). I, acclimatized; II, nonacclimatized; A, before an ascent; B, after the ascent.

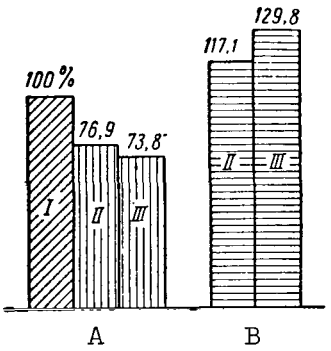

Figure 5. Change in time of restoration of oxygen saturation of the blood. A, before an ascent; B, after the ascent; I, original level (first 24 hours in camp); II, acclimatized; III, nonacclimatized.

where A_p is the rate of decrease in oxygen saturation of the blood in seconds (percent), 96 is the original oxygen saturation of the blood (percent), R_{oxy} is the minimum oxygen saturation of the blood at the end of the hypoxic phase, and T_p is the time in seconds during which the oxygen saturation of the blood drops.

Return to the original level of oxygen saturation after the hypoxic test during the stay in camp occurred sooner in the acclimatized persons and was slower in the nonacclimatized, as compared with the original values (fig. 5).

Judging by the degree of restoration of oxygen saturation of the blood to the original level after the hypoxic test, it was evident that the acclimatized subjects had adapted better to altitude. During the second examination (after

6-15 days in camp), the time of restoration of the original level of oxygen /85
saturation before an ascent was significantly less than during the first examination. After the ascent the rate of restoration increased even more. Among the nonacclimatized, the time of restoration of the original level of oxygen saturation (at the same times of examination) after the hypoxic test increased, on the other hand, both before and after an ascent. Consequently, both groups with different degrees of acclimatization exhibited shifts in oxygen saturation of the blood in different directions after the hypoxic test.

In the course of investigations conducted in earlier years, we found that the hemoglobin concentration decreased in some subjects after physical exertion during an ascent. A review of the literature turned up only scattered references to the subject (ref. 7). The Hb concentration decreased insignificantly in the acclimatized. During ascents it decreased almost equally in both groups (in the acclimatized by 7.6 percent and in the nonacclimatized by 6.7 percent). This decrease was probably due to fatigue during the ascents. The nonacclimatized also experienced fatigue. The Hb concentration might well have been greater in the absence of fatigue. It will be noted that, at the beginning of the stay in camp, the Hb concentration in the acclimatized was /86
87.7 percent, as compared with only 76.1 percent in the nonacclimatized. At the end of the stay (19th or 20th day), it was 85.5 percent in the acclimatized and 85.3 percent in the nonacclimatized.

The handwriting during the hypoxic test revealed almost identical changes in both groups in the first examination. After a climb, the handwriting improved slightly in both, with almost no differences between them. It is likely that the greater resistance of these functions to hypoxia was due to athletic training rather than to acclimatization.

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INVESTIGATION OF THE TRANSMISSION CAPACITY OF THE HUMAN VISUAL SYSTEM

A. A. Nevskaya

When man is a link in an automatic control system, it is important /87
to know the characteristics of his information transmission capacity, i.e., what quantity of information he can receive and process per unit of time.

We investigated the information transmission capacity (ITC) of the visual analyzer, which can be determined from the rate at which a human being perceives various images whose information content is known. Strictly speaking, the ITC is determined as the maximum rate of information transmission along a communication channel. However, not knowing the information-coding mechanisms in the visual system, we cannot say for which objects and tasks this maximum can be attained. It is necessary to investigate the time characteristics of visual reception for different assignments and conditions of observation. In the present work we have determined the rate of transmission and processing of information related only to evaluation of the shape of silhouetted objects and geometric designs.

Our task was to determine the amount of information received by an observer at various object-display times; to investigate the manner of ITC dependence on certain properties of the pattern and to determine the speed of perceiving solitary objects as a function of selection conditions (i.e., the information content of the pattern), pattern complexity and optical characteristics (brightness, angular dimensions, screening).

Various patterns were shown to the test subject for an exactly determined time (10-600 msec). The device for presenting them has been described earlier (ref. 5). The subject had to recognize and name the pattern shown. In order to limit the recognition time, it is necessary that after a showing its /88
traces are not preserved in the operative memory. For this we provided "image erasure": at the moment the object was removed from sight it was replaced by an "everyday" pattern. We determined the time required for correct recognition of images, or the average quantity of information received by the test subject for a given duration of pattern display.

The amount of information needed by the subject to identify the image correctly was determined by a priori probability for a given object. We utilized collections of 2, 3, 4, 5 or 10 pictures of objects or straight lines in 2, 4 or 8 possible positions. The subject's recognition training for each collection went on until recognition speed was not constant (each picture was shown several hundred times at various durations).

With small display times the test subject did not always identify the pattern correctly. In this case the information (I) received by him in a single presentation can be expressed as

$$I = H(x) + H(y) - H(x, y),$$

where H(x) is the entropy of the distribution of the probabilities of various patterns x: $H(x) = -\Sigma p(x) \log_2 p(x)$;

H(y) is the same for various responses of test subject y: $H(y) = -\Sigma p(y) \log_2 p(y)$;

H(x,y) is the same for all possible combinations of stimuli x and answers y: $H(x,y) = -\Sigma p(x,y) \cdot \log_2 p(x,y)$.

ITC was determined from the amount of information I received by the observer in a given time t: ITC = I/t. The test subjects were healthy males and females aged 16-38 years.

The pattern-recognition time was determined for objects known to all subjects from daily experience. The pictures appeared one at a time in the central area of the field of vision. They were visible to the subject at an angle of 4°, while the thickness of their constituent lines was about 3', such that the entire image fell in a field of clear vision and all its details considerably exceeded the resolution capability of the eye. Each test subject had to recognize 30 pictures.

The average time required for correct identification of such pictures, when the subject did not know what might be shown, ranged from 60-170 msec in different persons. Considering that the choice is made from 1,000 possible objects and that on the average 10 bits are necessary for correct identi- /89 fication, we obtain an ITC of 60-165 bits/sec. This value is approximate, since we do not know the active a priori probabilities of given patterns for a given observer.

If the subject was trained to recognize a limited collection of patterns, the time required for correct identification of all pictures diminished. The smaller the number of pictures in a collection n, the shorter the time spent in identifying them. The ITC of the observer can be evaluated as the minimum time in which all pictures are correctly identified, reckoning that, for correct identification, $\log_2 \cdot n$ information is required. We must remember that these definitions apply for complete subject training, i.e., when further training does not reduce correct-identification time.

Figure 1 shows the average amount of information received by a subject in one presentation of a pattern as a function of presentation time. The data relate to four test subjects and to collections of 3, 4, 5 and 10 pictures (same objects) chosen at random. Examples of these collections are shown. In each case the subject was well trained for the given collection.

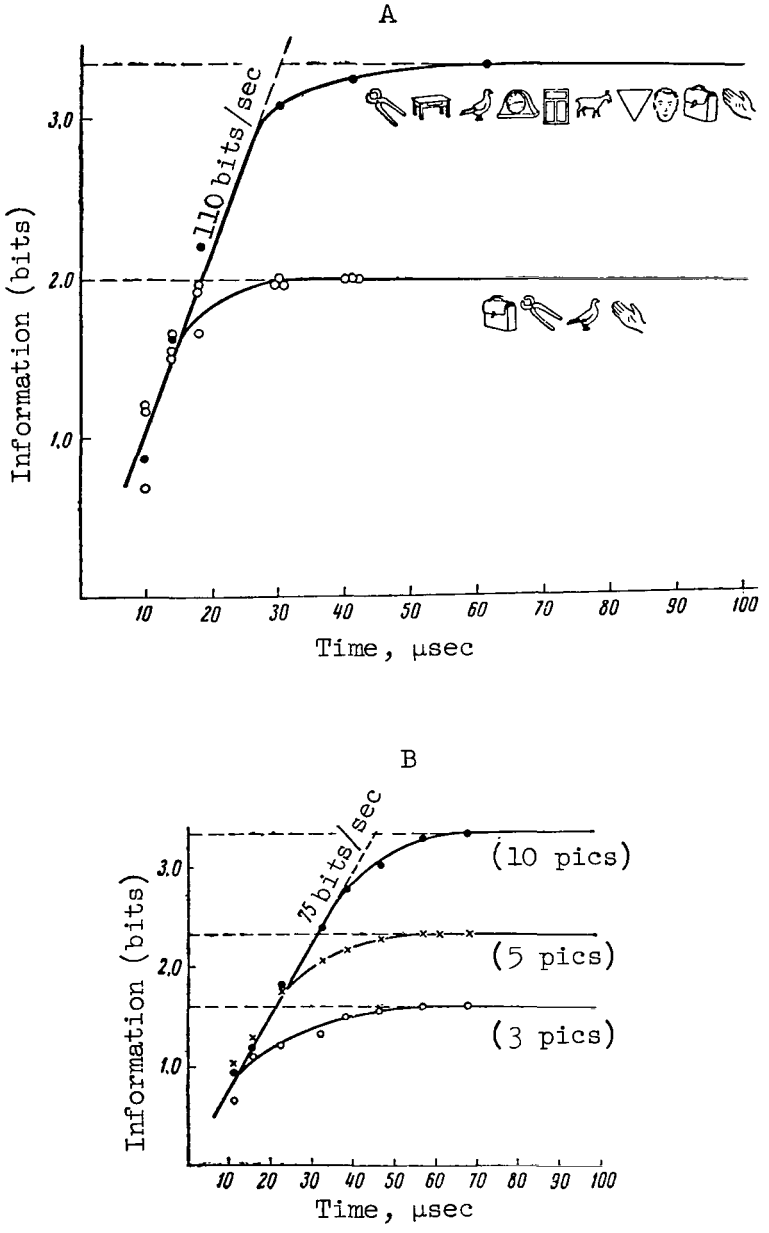
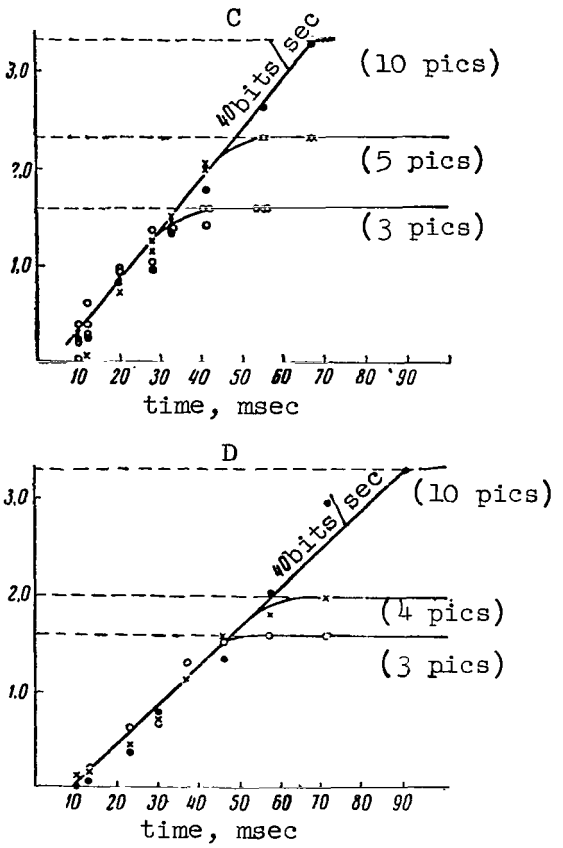


Figure 1. Dependence of quantity of information received during pattern recognition on exposure time for various groups of patterns. A, B, C and D are for subjects Ch. G., K. G., O. Ye. and Ya. V., respectively.



When viewing time was long, recognition was errorless and the amount of information received by the observer corresponded to the amount transmitted. As display time was reduced, the quantity of transmitted information decreased, but the amount received in a given time remained much the same, independently of the number of patterns in the collection. The slope of the information-quantity-time curve determined the ITC of the observer. The curve was the same regardless of the number of objects from which a selection was made. The test subjects' ITC, evaluated in this manner, ranged from 35-110 bits/sec.

These results, like the data of V. D. Glezer, I. I. Tsukkerman and T. M. Tsykunova (ref. 3), show that pattern recognition is determined not only by the properties of a given concrete object, but by the entire combination of visual models comprising the group shown a given observer. Studying the ITC of the visual system, we investigated at the same time the possibilities of its functional development and relation to changing conditions in the external world. We see that when there is a change in the probabilities of appearance of several objects a person is capable of mastering these probabilities and rearranging the visual-model coding system, such that only those clues to /92 a pattern are distinguished and transmitted which bear information, i.e., which permit him to make a choice in the system of possible visual models and to recognize the pattern (ref. 2). Objects whose appearance in a given situation is most probable (i.e., they carry little information) are recognized more rapidly than patterns with low probability. Because of such effective statistical

TABLE 1. ITC OF THE VISUAL SYSTEM OF SEVERAL OBSERVERS, DETERMINED BY VARIOUS METHODS.

Test subject	ITC, bits/sec: Determined with an unlimited group of visual objects	Determined after training on limited group from n patterns: According to time required for accurate identification	Determined after training on limited group from n patterns: According to rate of information receipt
Ya. V.	115	37 (n = 10); 29 (n = 4); 32 (n = 3)	40
O. Ye.	88	46 (n = 10); 43 (n = 5); 35 (n = 3)	49
K. G.	83	51 (n = 10); 42 (n = 5); 32 (n = 3)	75
Ch. S.	165	83 (n = 10); 54 (n = 4)	110
S. V.	57	42 (n = 10); 30 (n = 4)	45
G. N.	89	47 (n = 10); 33 (n = 5)	48
B. P.	103	33 (n = 4)	
S. S.	67	30 (n = 4)	
I. V.	101		
Average	96		61

coding, the visual system is capable, during solution of various tasks, of transmitting and processing information with approximately the same speed as its ITC.

Table 1 shows ITC values determined when the subject was unaware of what pictures might be shown (after training on a limited collection of patterns), arranged according to correct-identification time and to the slope of the viewing-time--information-received curve.

The ITC values obtained by all estimates are of the same order. Divergences in ITC values are evidently caused by peculiarities in the methods used for measurements.

The ITC obtained with an unlimited collection is always higher than with a restricted pattern collection, which may be explained in various ways. Required recognition time can be divided into two parts: first, the time /93 spent accumulating photic/light energy for separation of the useful signal from noise and processing information in the retina, as a result of which the outline of the image is distinguished, possibly along with certain elementary clues about

it; and second, the time given over to recognition proper, i.e., choice of a decision as to which category to assign the object shown.

Understandably, limitation of the group of visual models may affect only the second part, and therefore the relative fraction of time for signal formation is the greater as the group of possible patterns diminishes. Besides, it is possible that among the objects presented for recognition under conditions of unlimited choice, those predominated which were frequently met in the subject's daily life as opposed to those encountered less often, with the result that the average time for recognition of a single picture appeared to be reduced.

If we determine the ITC in terms of the time required for errorless recognition of a limited selection of images, then somewhat smaller values are obtained than those derived from the slope of information--exposure-time curves. As is apparent from figure 1, this occurs because of isolated mistakes during the time which--judging from information-transmission speed--should be sufficient for accurate identification; i.e., the visual system is not a wholly ideal system of communication.

As was shown earlier (ref. 1), deviations from an ideal system increase as the group from which the visual system is working decreases. This is a consequence of the fact that in an ideal communication system optimal statistical coding must be accomplished, i.e., coding by large blocks. The fewer the patterns in the group, the fewer the number of useful clues that aid in their recognition and, consequently, the smaller the possibilities of coding by large blocks. For this reason, in determining the ITC by time to correct identification of objects from very small collections of patterns, we obtain reduced values.

Is the concept of an ITC applicable for all types of objects recognized? On the basis of theoretical considerations we might hypothesize the existence of systems which automatically respond when a given configuration appears in the field of vision. In references 6 and 7 the authors, who made electrophysiological experiments on cats and frogs, discovered in the higher visual centers of these animals cells which are excited if a given configuration (e.g., a line with a certain slope moving in a given direction, or an arc of given curvature) strikes the receptors connected to them; however, they either do not react at all or respond weakly to a different configuration.

On the basis of these and some electrophysical data it was suggested in references 1 and 2 that a human's discovery of such simple configurations as straight lines, angles and arcs--i.e., the basic elements comprising /94 diverse complex patterns--occurs by innate mechanisms, which operate in the manner of elementary automatic devices. In this case the simplest configurations would have to be identified in a constant time independent of the probability of the objects.

In our experiments the time characteristics of the process of recognizing complex visual models (contoured patterns of objects) were compared with simple configurations (straight lines of various slopes). The line appeared in the

central area of the field of vision, with a length of 3° and a width of 2'40". The test subject had to determine how the line was situated.

In the first experiments we obtained for line recognition about the same ITC values as for recognition of complex objects (ref. 1). If the test subject did not know what might be shown, approximately 100 msec--about the same time as for unknown object patterns--was required for accurate recognition. Occasionally the subject noticed a line at lower durations, but was not able to say whether it was a single line of part of some more complex pattern.

When the subject knew that a line might be shown in various definite positions, the recognition time diminished. The amount of information /95 was about the same as with pattern recognition. With longer display times the lines were recognized somewhat less well than were complex images (fig. 2A). We explain the fewer errors in identifying the latter by the fact that their recognition can be accomplished from a large number of closely juxtaposed

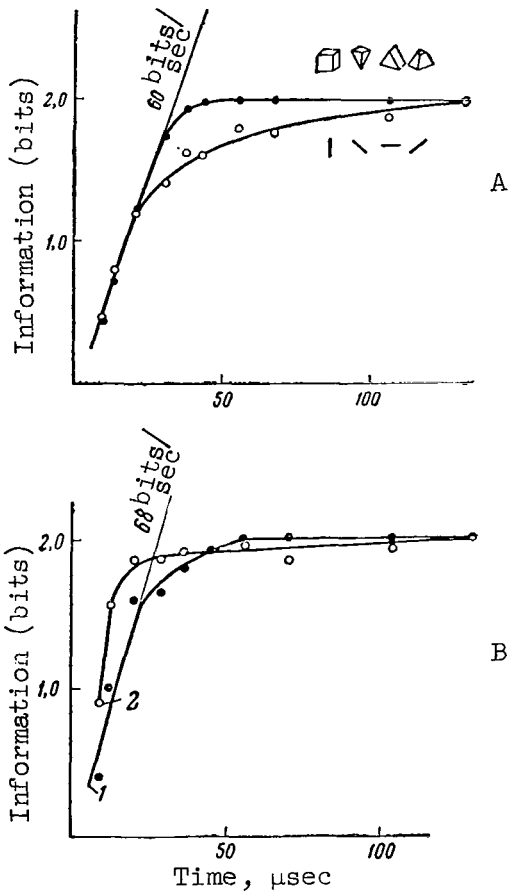


Figure 2. Amount of information received as function of exposure time for different pattern complexities (subject O. Ye.). A and B are for slight and prolonged training, respectively.

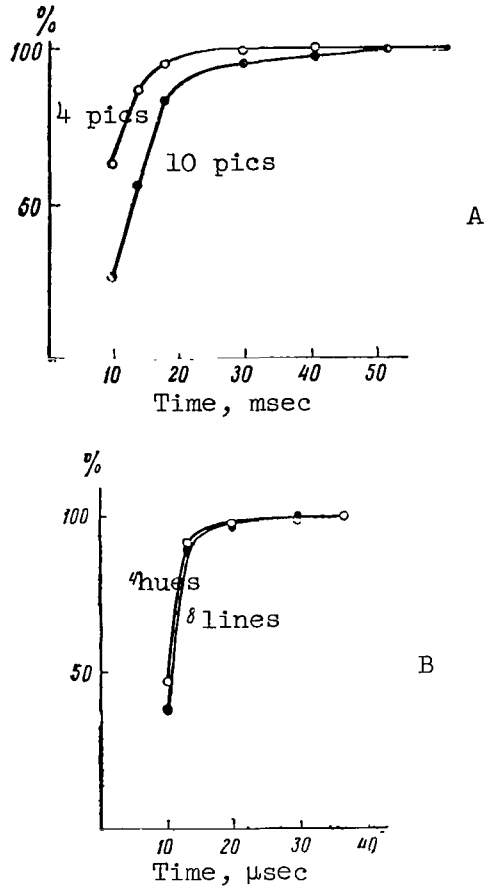


Figure 3. Probability curves for accurate pattern recognition of objects (A) and lines (B), given various groups, as function of viewing time (subject Ch. G.).

"stand-by" lines (i.e., these patterns possess a large statistical redundance), and the error made on one clue can be made up by correct choice of another.

But with further training (although, it would appear, identification of such a simple object does not require training, the observer not needing to separate and remember clues distinguishing one pattern from another), after several hundred repetitions the lines began to be recognized considerably better at small presentation times than complex pictures (fig. 2B). The information-receiving speed at short display times reached several hundred bits/sec. After prolonged training we succeeded in obtaining in several test subjects a line recognition that was identical, whatever the number of possible positions.

Figure 3 shows how the probabilities of accurate identification of objects and lines change with an increase in the view time, when the human subject

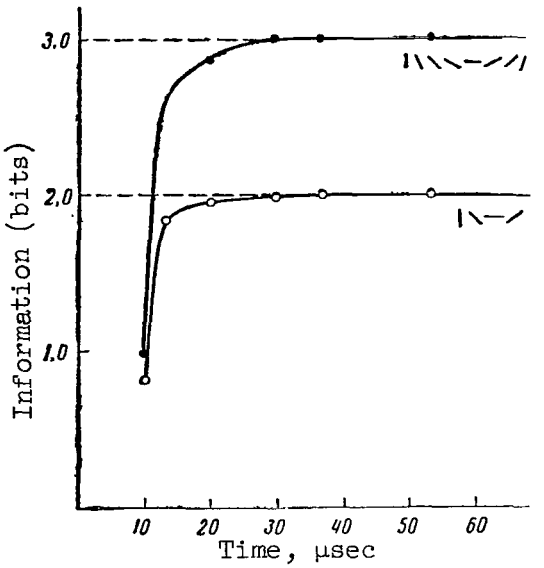


Figure 4. Dependence of amount of information received during line-slope testing on time of exposure of various images (subject Ch. G.).

has been well trained on various numbers of images. The pictures were always recognized more easily when the number of possible alternatives was small. /96 This situation did not change, whatever the degree of training. The probabilities of line identification turned out to be almost identical in an experiment in which the line could occupy 4 or 8 different positions.

This shows that information received in the same viewing time is always greater when the choice is made from 8 possibilities than when it is made from 4 (fig. 4), i.e., the recognition speed is almost independent in this case of the information content of the images. These results provide support for the proposition that recognition of very simple configurations occurs not by successive choice among a certain number of possibilities, but that there exist automatic and apparently innate mechanisms which react in the manner of an unconditioned reflex to elementary clues about the pattern. The ITC concept cannot be applied to systems of such kind.

The task of the next experiments was to investigate in what ways the ITC is affected by such factors as pattern dimensions, line width, brightness and screening. We determined the correct-identification time for complex patterns in all cases. Since in these experiments the test subject was presented with a small number of images for identification (3-10) for a single gradation of the factor being studied, the ITC value could not be calculated with reliable precision.

Furthermore, in several variants of the experiments, in order to /97 reduce the variability of the data, we repeated presentations of the same object

under different values of the factor being studied. Although such repetition (up to 10 times) is insufficient for total carry-over to a new abbreviated group, nonetheless in certain cases it did somewhat shorten the recognition time. Then, reckoning that identification occurs with an unlimited group, we would obtain enhanced ITC values; but, assuming that the subjects were working from a restricted group, we would be able to reduce those values. Therefore, we will not cite absolute values for ITC. Here we are concerned only with ITC changes under the effect of the factors mentioned.

The time required for recognition of patterns and consequently the ITC of the visual system change within certain limits as a function of the dimensions of the patterns shown.

To determine this we showed 10 test subjects 20 various complex images each, with 5 different angular dimensions (8, 4, 2, 1 and 0.5°) and average line widths (6, 3, 1.5, 0.75 and 0.366'). Presentations were made in random order, and the subjects did not know which pictures would be shown.

In another variant the subjects were informed that they might be shown one of three patterns, each picture being shown in each of its five dimensional variations. The order of showing was random, so that when the data were averaged possible effects of training would be levelled out. This variant of the tests was conducted on 13 subjects, who were not trained on a given collection of patterns; therefore, although the selection was formally abbreviated, the time necessary for identification did not change substantially (125.5 msec average in the first variant and 118.9 msec in the second). Only the variability of the data became smaller (σ_1 = 90 msec, σ_2 = 73 msec; difference reliable within

$p < 0.01$).

Figure 5 presents the results obtained. In both variants the recognition time increased as the pattern dimensions decreased. With a reduction to one-half size the average time rose 1.33 times. Obviously, the ITC dropped in the same degree.

In treating the data by dispersion analysis the following factors turned out to be of significance: pattern size, picture, test subjects and picture--dimension and dimension--subject interaction (the last factor only in the first variant).

It remained unclear what produced the ITC drop: a reduction in /98
the overall size of the pattern or a reduction in the width of constituent lines. In the following series of experiments we determined recognition time on pictures of 8, 4, 2 and 1°, while in each case retaining a line width of 1.2'. Sixteen subjects were used, each of whom was shown in random order pictures of nine different objects in each of the four sizes.

In this case the identification time was minimal when the images were visible in the 2-4° dimension (fig. 6), growing somewhat (22.6 percent on the average) when the size was reduced to 1°. With an 8° angle the average recognition time rose by 3.8 percent. Evidently this is explained by the fact

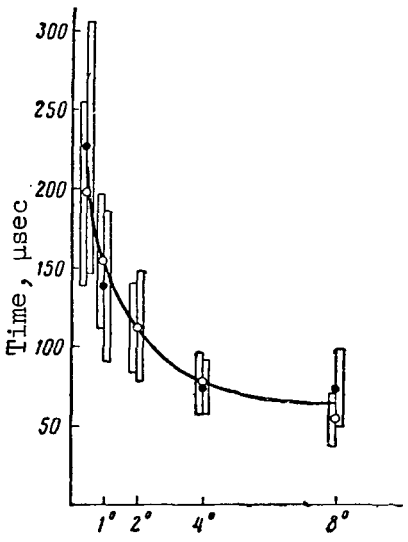


Figure 5. Effect of picture dimensions and line widths on recognition time. Vertical sections designate data scatter for different observers (±σ) in two variants of experiment. White circles represent test with repetitions of 3 pictures; black circles, 20 pictures.

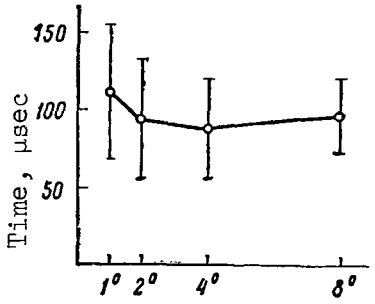


Figure 6. Effect of picture size on recognition time when line width is constant. Vertical strokes designate data scatter for different observers (±σ).

that the pattern in the last instance did not fall entirely in the zone of clear vision. All differences were small: with an eightfold change in picture dimensions the average time did not change by more than 27 percent. Data obtained on various test subjects were more varied as pattern dimension decreased. Dispersion analysis showed that in this case the following factors were significant: test subjects; pattern size; picture; dimension--picture, /99
dimension--subject and picture--subject interaction.

When we compare the data of the previous series with those just described, we learn that for the most part the increase in identification time with a decrease in dimension is the result of changes in the width of a pattern's constituent lines, rather than a change in the dimensions of the picture as a whole. We can conclude that the ITC to a large extent depends on line thickness, but hardly changes within pattern dimensions of 1-8°.

Glezer, Tsukkerman, and Tsykunova (ref. 4) have established that at low-brightness levels the ITC grows linearly as the logarithm of brightness as a result of transformation of the volume of the visual channel and enhancement of visual acuity. With large brightness values, when visual acuity no longer limits pattern recognition, the ITC of the visual system becomes independent of brightness.

This result has been corroborated in the present investigation by determination of recognition speed at various brightness levels in the field of vision.

We determined in one subject the effect of brightness on time of recognition for pictures of 24 objects of various sizes (8, 4, 2 and 1°) and line widths (6, 3, 1.5 and 0.75', respectively). Each picture was shown 10 times in the course of the experiment at various brightnesses and dimensions, the viewing order being random. The brightness of the field of vision varied from 0.01-1,000 nit and recognition time was determined for 10 different pictures of each dimension at 6 brightness levels differing by an order of magnitude. The data of this experiment are shown in figure 7.

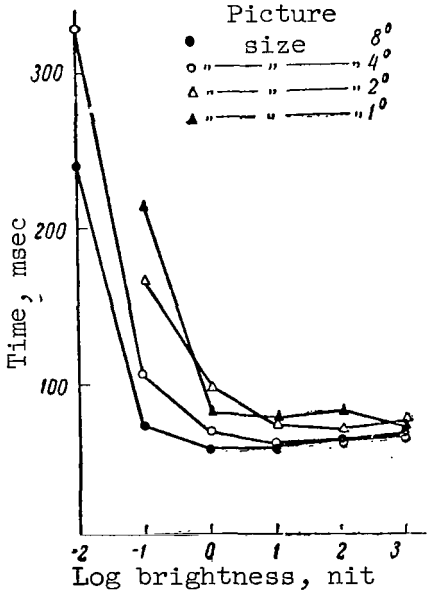


Figure 7. Effect of brightness on pattern recognition time when both picture size and line width vary (subject N. A.).

Changes in brightness from 1,000 down to several nit did not affect picture recognition time. When brightness was reduced still further, an increase in recognition time was observed, beginning with pictures of small size and continuing up to larger ones. In a single observer high-brightness levels did not significantly alter recognition time when dimension was changed, but these differences increased when brightness declined, until at 0.01 nit pictures of small size and narrow line width could not be identified.

According to these data, the ITC of the visual system does not depend on brightness, provided the latter exceeds a certain threshold (several nit for pictures of small dimension and several tenths of a nit for larger pictures with heavier lines); ITC does drop when brightness declines below this threshold. This relationship is associated with a reduction in visual acuity. Evidently, a decline in pattern brightness, just as in the case of line-width /100
reduction, affects ITC, increasing that part of recognition time spent in accumulating and distinguishing a signal from noise.

Screening of the patterns presented was effected by introducing randomly distributed black dots with a size on the order of picture line width (3'). Accurate identification time was determined for various numbers of dots per

cm^2 of diapositive, which corresponds to a circle with a diameter of 4^o, the size of the pictures presented (0-400 dots). The average brightness of the field of vision in all cases was about 50 nit. The bright areas of the pattern were highlighted when the dots were introduced, but as we have shown, a change in brightness with such limits does not influence the ITC of vision. The tests were run with six subjects, of whom three were familiar with the patterns presented.

As shown in figure 8, recognition time does not change when dot density is 100-200/cm^2. It is notable that when viewing time was sufficient /101

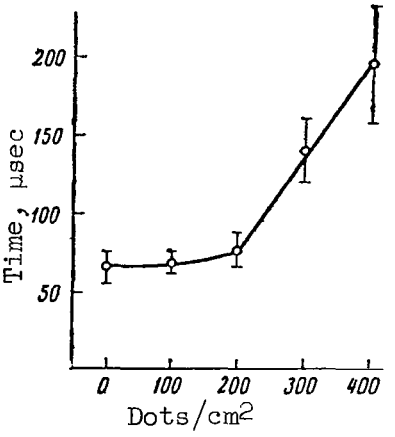


Figure 8. Effect of confusion on pattern recognition time. Vertical strokes designate data scatter for different observers (±σ).

for accurate identification, the subjects usually did not notice the screening background. With a further increase in dot density, identification became difficult and its time increased.

Thus, as with brightness, ITC remains unaffected by screening up to a certain threshold, after which it drops rather sharply.

Both factors decrease the signal-to-noise ratio. The quality of preserving an information transmission capability with a change in the signal-to-noise ratio up to a certain limit, and the presence of a sharp threshold effect, are characteristic of ideal communication systems.

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PECULIARITIES OF HUMAN AUDITORY SENSITIVITY UNDER CONDITIONS OF CONTINUOUS AND PROLONGED MEDIUM INTENSITY NOISE

Yu. V. Krylov

The operation during weightlessness of various life-support systems /102 requires the continuous operation of fans which are the principal source of noise in spacecraft cabins during the free-fall phase of flight. Under certain conditions this noise may have an adverse effect on the cosmonaut. Despite a considerable number of reports on various aspects of the adverse effects of noise (refs. 1, 2, 6, 9, 11, 13, 14, 21 and 23), the question of the effect on man and on the human auditory function of continuous exposure for several days to medium-intensity noise so far remains unstudied; the longest continuous exposure to noise has not exceeded 8-12 hours.

The practical value of those studies which have been conducted lies in their physiological evaluation of the auditory function to determine the capacity for reliable reception of auditory information after several hours of continuous exposure to medium-intensity noise. The present article generalizes the results of auditory sensitivity tests made on 8 subjects, who remained in a small sealed chamber for 27-60 days.

During this entire period the subjects were exposed to noise in a free acoustic field. Maximum noise intensity was 60-65 db at frequencies of 800-1,800 cps, the intensity dropping by 4-5 db per octave as the high-frequency end of the spectrum was approached.

The audiometer method was employed. Acoustic signals from the output of an AU-5 audiometer were transmitted to the test subject in the chamber by a telephone with hermetic earpieces. The telephone had a baffle which reduced external interference by 7-8 db in the lower frequencies. The subject made his responses with a button controlling the signal light of the audiometer. He was warned of the importance of uniform arrangement of the headset to /103 avoid error during signal reception. The audiometer had a special attachment to avert formation of a time reflex.

Auditory thresholds were investigated at frequencies in the range 125, 250, 500, 1,000, 6,000 and 8,000 cps. In order to obtain precise data, auditory sensitivity was measured at least 3 or 4 times prior to the experiment. During the testing period, the investigation proceeded according to a standard format. At the end of the experiment the determination of thresholds continued until the subjects had fully regained their auditory function. In all, more than 350 auditory tests were run during the experiments.

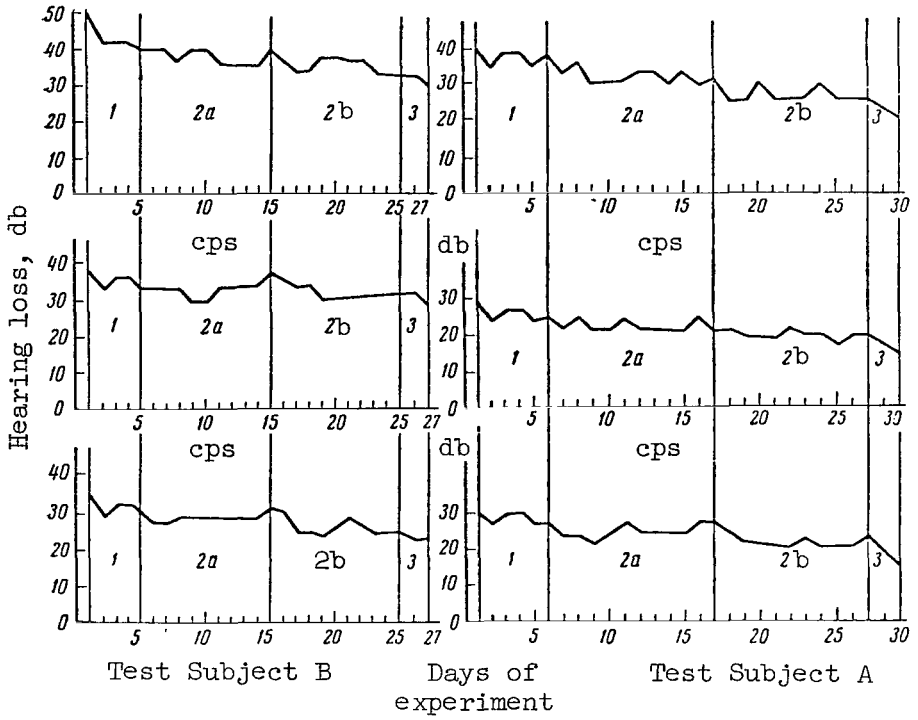

Figure 1. Dynamics of auditory sensitivity at frequencies of 250, 1,000 and 3,000 cps during prolonged sequestration of subjects in small chamber under conditions of continuous noise. 1, 2a, 2b and 3, experimental stages.

In evaluating the results it is necessary to take into account the /104 fact that the noise level remained constant throughout the experiments, and that the experimental conditions in general were held quite uniform. When the auditory function is studied under such conditions, dynamic changes in threshold parameters during the experiments as a whole are especially significant.

Determination of auditory thresholds during exposure to noise is essentially a study of masking. Since noise intensity is not uniform along the spectrum, the increase in auditory thresholds at various frequencies will not be the same; this is in agreement with data in the literature (refs. 7, 16 and 17).

Analysis of the experimental materials pointed out a certain peculiarity of the curves expressing changes in auditory sensitivity during the experiment. As the figure shows, threshold changes can be classified into three stages.

The first of these stages is marked by maximal increase in the auditory threshold, particularly during the first day of the experiment.

According to contemporary concepts of the phenomenon of masking (refs. 7 and 19), this increase in the auditory threshold is related to the masking effect of noise and should be regarded as a function of changes in the noise characteristic. However, since the frequency and amplitude components of noise did not change during the entire experiment, the masking effect could not diminish; therefore, the maximal auditory threshold increase observed on the first day must obviously be due not only to adaptive changes in the auditory sensor, but also to the total reaction of the test subject to the novelty of the situation and to the new conditions of his vital activity.

In the next 4-5 days of the experiment, the threshold drops by 5-7.5 db in comparison with the first day. Evidently these days are marked by a unique sort of "habituation" to the experimental conditions, which had a highly pronounced cyclic character. Adequate stimuli for the auditory sensor being sufficiently uniform, there occurs a sort of "readjustment" or "returning" of the activity of the sensor (ref. 8). This stage may be called the period of initial adaptation of the human being to the new conditions of the acoustic environment, which has now become constant for him.

The second stage embraces the major period of the experiment (23 to 24 days for 30-day tests) and is characterized by considerable stability of auditory sensitivity (2a and b on the graph).

I. P. Karagodina has shown (ref. 10) that a 2-7 hour exposure to noise, whose intensity and spectrum approximate those of the experiment, produces changes in a number of the functions of the organism, including hearing. On the first day of exposure a drop in auditory sensitivity was actually observed, after which stabilization occurs, with some later improvement; this improvement was a somewhat unexpected factor, since continuous exposure /105 to noise had already gone on for many days by that time.

This state of the auditory function can be understood only as a phenomenon of the general adaptation of the organism to a new auditory environment and cannot be explained by modern concepts of the function of Corti's organ (refs. 14, 24 and 25). There can thus be no doubt that the higher sectors of the central nervous system participate in the phenomenon described here (refs. 2, 5, 8 and 12). At the present time we also cannot discount the role of certain afferent paths reinforcing the auditory function (ref. 18).

The third stage occurs during the last days of the experiment and consists of a further lowering of the auditory thresholds. This improvement cannot at present be fully explained. Evidently it is related to the considerable anticipation by the subjects of the end of the experiment and in this case can be explained by reinforcement of the vestibular process in the cerebral cortex. This phenomenon has been described by some investigators studying the behavioral reactions of test subjects in lengthy experiments (ref. 20) and has been termed the "last twitch."

Restoration of auditory sensitivity occurs in the 10-15 min following cessation of noise. Repeated tests of auditory sensitivity for several months after the experiment showed that the subjects had fully regained the auditory function.

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SOME DATA ON THE ANIMAL LINK IN A CLOSED ECOLOGICAL SYSTEM

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Most specialists in space nutrition now believe that extended /107
flights (up to several years) are feasible only if use is made of a closed system with the recirculation of substances to permit reduction in weight and size of the vehicle's cabin (refs. 14 and 15). This seems to be the basis for solving the problem of feeding the crews serving on long-term orbital and planetary stations.

One of the most difficult elements in a closed system is a reliable food cycle. At the present state of our knowledge, the most practical approach is to include unicellular algae, higher plants, animals and man in the system. Unicellular algae and higher plants can be used for biological regeneration of air, water purification and as food for the animals and food for the cosmonauts. Foreign authors propose goats, pigs, rabbits, etc. The best animals for this purpose are the species that have been studied from the standpoint of zootechny, physiology and nutrition and that can be maintained most economically, e.g., chickens, ducks, rabbits, etc.

From a review of the literature and our own calculations, we have tentatively concluded that chickens and ducks are the most suitable for space- /108
flight. Several considerations led to the choice of these fowl. Fowl produce highly nutritious and tasty meat and eggs, show a high weight increase per unit of feed and grow rapidly. Chickens and ducks can be fed unicellular algae, man's food wastes and wastes from slaughtered fowl (viscera, processed bones, feathers and eggshells). In addition, offspring can be produced by incubating the eggs. Thus, in case the brood stock dies, the flock can be renewed if a certain number of eggs are available. The efficiency of breeding fowl for meat (table 1) can be judged from the many offspring that are produced in a short period of time (table 1, from ref. 4).

It is evident from the table that the offspring of chickens and ducks are the heaviest. The following amount of edible meat and protein (in kg) is obtained per 100 kg of meat (live weight): fowl meat, 58.0, including 11.6 kg of protein; beef, 45.0, including 8.1 kg of protein; sheep, 42.0, including 6.3 kg of protein; swine without hide, 60.0, including 8.4 kg of protein.

Protein is most abundant in fowl meat. It is also superior qualitatively, as shown in table 2 (ref. 12).

TABLE 1. WEIGHT OF OFFSPRING OF VARIOUS ANIMALS.

Index	Sow	Hen	Duck	Goose	Turkey
			Brood stock		
Total weight of adult animals, kg	100.0	100.0	100.0	100.0	100.0
No. of animals	1	50	40	20	16
Weight of 1 animal, kg	100.0	2.0	2.5	5.0	6.0
			Offspring		
No. of adults from 1 adult animal	12-16	50-80	25-50	10-12	20-24
No. of animals from all adult animals	12-16	2,500-4,000	1,000-2,000	200-240	320-380
Average weight of 1 offspring, kg	100	1.0	2.5	5.0	5.0
Total weight of offspring, 100 kg	12-16	25-40	25-50	10-12	16-19

The economic utility in raising chickens can be judged from the figures in table 3 (ref. 4).

It is evident from table 3 that to produce 1 kg of broiler meat and fat (no bones) requires the consumption of half as much feed as it does to produce beef and about two-thirds as much to produce pork.

The size of the flocks is determined by the animal protein requirements of one person. A cosmonaut's diet must be adequate quantitatively and qualitatively. It should contain approximately 3,000 kcal. The proportions of proteins, fats and carbohydrates must be optimal, namely: proteins should make up 13-15 percent of the total calorie content of the daily diet; fats, 25-30 percent and carbohydrates, 55-60 percent.

TABLE 2. QUALITATIVE COMPOSITION OF PROTEIN IN MEAT FROM VARIOUS ANIMALS.

Kind of meat	Of total amount of protein in meat (%)		
	Nutritious (albumin)	Non-nutritious (elastin and collagen)	Ratio of non-nutritious to nutritious protein
Fowl	19.5	1.5	7.7
Beef	18.0	3.0	23.0
Swine	6.0	5.0	83.3

TABLE 3. CONSUMPTION OF FODDER UNITS (IN KG OF CORN) PER KG OF PRODUCTION.

Species of animal	Per kg of live weight	Per kg of meat and fat without bones	Per kg of protein
Cattle and calves	5.8	13.2	84
Swine	4.9	7.8	90
Chickens (broilers)	3.6	6.7	34
Turkeys	5.2	8.3	42

The nutrients should be distributed by weight about as follows: 104 g of proteins, 80 g of fats, and 447 g of carbohydrates. Animal proteins should make up 40-60 percent of the total.

The cosmonaut's need for animal products can be satisfied by fowl meat and eggs. The average chemical composition of chicken and duck eggs is shown in table 4.

An egg consists of 56 percent protein, 32 percent fat and 12 percent /110 shell. The size of the duck flock is based on the assumption that 40 percent of the total protein would be animal protein. The daily diet of one person should include 40-45 g of assimilable animal protein. This protein will be obtained by the cosmonauts from fowl meat and eggs. One duck egg provides approximately 8.4 g of assimilable protein. Consequently, 33.2 g more of protein has to come from meat. This amount of protein is found in 249.6 g of meat from first-grade ducks.

Ducklings of the Peking breed weigh about 1,420 g when forty days old. It is suggested that they not be slaughtered at sixty days, as is the usual practice, but at forty days because this is more sparing of oxygen and feed in a closed ecological system. Since the slaughter weight of ducks is 90 percent, while the edible part of prime carcasses is 68.8 percent, one duckling can supply the meat

TABLE 4. AVERAGE CHEMICAL COMPOSITION OF CHICKEN AND DUCK EGGS (IN %).

Egg	Content of					Calories per 100 g
	Water	Nitrogenous substances	Fat	Carbohydrate	Ash	
Chicken	73.67	12.57	12.02	0.67	1.07	158
Duck	70.81	12.77	15.04	0.30	1.08	184

needs of a cosmonaut for 3-4 days. Consequently, nine such ducklings would have to be slaughtered every month. In view of the natural loss of ducklings, it is best to count on ten animals. To obtain ten ducklings with an egg fertilization rate of 89-90 percent and a hatching rate of 61 percent, 18 eggs are necessary.

Egg-yolk oil is included in the ration of ducklings until they are 10 days old, with 2.2 eggs needed for this purpose. Since a cosmonaut will eat thirty eggs a month (1 a day), a total of fifty eggs will be required. They can be obtained from six adult ducks laying an average of 8-9 eggs a month.

The turnover of the flock is calculated in the light of the foregoing (table 5). Ten ducklings are hatched the first month. Allowing for natural loss, by the 40th day nine ducklings are alive (cf. second month), eight of which are slaughtered, while one is raised until it is seven months old, then to replace an adult duck which is slaughtered. Nine ducklings are hatched the second month, eight of which are kept until they are forty days old, after which /111 they are all slaughtered. This group of ducklings is not set aside for renewal of the flock. Ten ducklings are hatched the third month, one of which is used to rebuild the flock, etc. Thus, one out of six adult ducks is replaced every month, while a drake is replaced once in six months. It is also evident from the table that the total monthly duck population is 29-30 head. Thus, it is clear that a cosmonaut's monthly meat supply can be provided by slaughtering eight 40-day-old ducklings and the adult fowl (six ducks and two drakes a year), which weigh (live weight) approximately 13.5 kg.

The daily feed and oxygen requirements and the amount of carbon dioxide released by a theoretical flock of ducks are shown in table 6. They will need 4.4 feed units per kg (daily average) and 1,285 liters of oxygen. The amount of carbon dioxide released by the entire flock will average 1,038 liters a day.

The calculations of the maintenance of chicks are similar to those for ducks. The average weight of a chicken egg is 50 g; 100 g (the cosmonaut will receive two eggs) contain 9 g of assimilable protein. Consequently, 32.6 g of assimilable protein will have to be supplied by meat. This amount of protein is present in 187 g of prime-grade chicken meat. Broilers will be /114 slaughtered when they are 70 days old with an average live weight of 1,030 g (the edible part of the carcass weighs 602 g).

To supply a cosmonaut with animal protein will require 9.5 kg of chicken meat in live weight per month. This amount of meat can be obtained from eight

TABLE 5.* TURNOVER OF A FLOCK OF DUCKS BY MONTHS NEEDED TO SUPPLY 1 COSMONAUT WITH ANIMAL PROTEIN.

Age	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I
1st day	10	9	10	9	10	10	10	9	10	9	10	10	10
40 days	9	9	8	9	8	9	9	9	9	8	8	9	9
2 months	1	1c	1	—	1	—	1	1c	1	—	1	—	1
3 "	—	1	1c	1	—	1	—	1	1c	1	—	1	—
4 "	1	—	1	1c	1	—	1	—	1	1c	1	—	1
5 "	—	1	—	1	1c	1	—	1	—	1	1c	1	—
6 "	1	—	1	—	1	1c	1	—	1	—	1	1c	1
7 "	1c	1	—	1	—	1	1c	1	—	1	—	1	1c
Adult ducks	6	6	6	6	6	6	6	6	6	6	6	6	6
Drakes	1	1	1	1	1	1	1	1	1	1	1	1	1
Total	30	29	29	29	29	30	30	29	29	29	29	30	30
No. of fowl slaughtered	8+1	8+1	8+1	8	8+1	8	8+1	8+1	8+1	8	8+1	8	8+1

*1c designates duckling that will replace drake.

broilers and a hen slaughtered at culling time (six hens and two roosters a year). Ten chicks have to be hatched to produce eight broilers and renew the flock.

Some 78-80 eggs are required to feed a cosmonaut, hatch chicks and feed them when young. Seven adult layers can supply this many eggs. Table 7 shows the turnover of a flock of chickens.

It is evident from table 7 that the total monthly number of chickens is 37. One hen is replaced every other month; the rooster, every six months.

The daily feed unit and oxygen requirements of a theoretical flock of chickens and the amount of carbon dioxide released by them are shown in table 8.

It is clear from the table that the feed requirement of the entire flock averages 2.9 feed units per kg; oxygen consumption, 1,170 liters. The amount of carbon dioxide released by the entire flock averages 1,046 liters a day.

Table 9 presents data on the content of nutrients and caloric value of the products obtainable from a theoretical flock of ducks and chickens per month.

It is evident from table 9 that one cosmonaut will derive 44.6 g of /117
proteins daily from products obtainable from ducks, 78.1 g of fats, and

TABLE 6. DAILY FEED AND OXYGEN REQUIREMENTS OF THEORETICAL FLOCK OF DUCKS AND AMOUNT OF CARBON DIOXIDE RELEASED BY THEM.

	Live weight of 1 duck	1st month				2nd month				3rd month			
		No. of ducks	Feed units, g	Oxygen, liter	Carbon dioxide, g	No. of ducks	Feed units, g	Oxygen, liter	Carbon dioxide, g	No. of ducks	Feed units, g	Oxygen, liter	Carbon dioxide, g
1 day old	59	10	300	41,11	33.25	9	270	37.00	29.8	10	300	41,11	33.25
40 days old	1420	9	1800	514.8	498.95	9	1800	514.8	498.95	8	1600	499.84	443.40
2 months old	2130	1	233	78.89	74.78	1	233	78.89	74.78	1	233	78.89	74.78
3 " "	2600	—	—	—	—	1	208	86.73	82.99	1	208	86.73	82.99
4 " "	2760	1	183	83.95	72.92	—	—	—	—	1	183	83.85	72.92
5 " "	2800	—	—	—	—	1	183	64.26	48.82	—	—	—	—
6 " "	3000	1	223	63.06	40.68	—	—	—	—	1	223	63.06	40.68
7 " "	3000	1	223	63.06	40.68	1	223	63.06	40.68	—	—	—	—
Adult ducks	3000	6	1338	378.36	244.08	6	1338	378.36	244.08	6	1338	378.36	244.08
Drake	3500	1	223	64.43	41.3	1	223	64.43	41.3	1	223	64.43	41.3
Total	—	30	4523	1287.56	1046 69	29	4478	1287.67	1061.45	29	4308	1296.27	1033.40

Note: commas in tables represent decimal points.

Table 6 (Concluded)

4th month				5th month				6th month			
No. of ducks	Feed units, g	Oxygen, liter	Carbon dioxide, g	No. of ducks	Feed units, g	Oxygen, liter	Carbon dioxide, g	No. of ducks	Feed units, g	Oxygen, liter	Carbon dioxide, g
9	270	37.0	29.8	10	300	41.11	33.25	10	300	41.11	33.25
9	1800	514.8	498.95	8	1600	499.84	443.4	9	1800	514.8	498.95
—	—	—	—	1	233	78.89	74.89	—	—	—	—
1	208	86.73	82.99	—	—	—	—	1	208	86.73	82.99
1	183	83.85	72.92	1	183	83.85	72.92	—	—	—	—
1	183	64.26	48.82	1	183	64.26	48.82	1	183	64.26	48.82
—	—	—	—	1	223	63.06	40.68	1	223	63.06	40.68
1	223	63.06	40.68	—	—	—	—	1	223	63.06	40.68
6	1338	378.36	244.08	6	1338	378.36	244.08	6	1338	378.36	244.08
1	223	64.43	41.3	1	223	64.43	41.3	1	223	64.43	41.3
29	4428	1291.12	1059.55	29	4283	1273.8	999.23	30	4495	1275.81	1030.70

TABLE 7.* TURNOVER OF FLOCK OF CHICKENS BY MONTHS NEEDED TO SUPPLY 1 COSMONAUT WITH ANIMAL PROTEIN.

Age	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I
1st day	10	10	10	9	10	9	10	10	10	9	10	9	10
1st month	8	9	9	9	8	9	8	9	9	9	8	9	8
70 days	9	8	9	9	9	8	9	8	9	9	9	8	9
3 months	—	1	—	1	1п	1	—	1	—	1	1п	1	—
4 "	1	—	1	—	1	1п	1	—	1	—	1	1п	1
5 "	1п	1	—	1	—	1	1п	1	—	1	—	1	1п
Hens	7	7	7	7	7	7	7	7	7	7	7	7	7
Roosters	1	1	1	1	1	1	1	1	1	1	1	1	1
Total	37	37	37	37	37	37	37	37	37	37	37	37	37
No. of chickens slaughtered	9	9	9	8	9	8	9	9	9	8	9	8	9

*1п designates chick that will replace rooster.

0.39 g of carbohydrates with a caloric value of 577 kcal; from products obtainable from chickens: 41.5 g of proteins, 28.3 g of fats, 0.43 g of carbohydrates with a calorie value of 434 kcal.

The caloric value of the feed consumed by the flocks during a month is 439,355 kcal for ducks and 289,338 kcal for chickens. Thus, 25.4 kcal of feed is used for ducks per kcal of obtainable products, 22.2 kcal for chickens. Oxygen consumption by ducks and chickens per kcal of obtainable products is 2.2 and 2.7 liters, respectively, with ducks releasing 1.8 liters of carbon dioxide and chickens releasing 2.4 liters.

Thus, our calculations show that 3.2 kcal less feed is used for chickens per kcal of obtainable products than for ducks, but the chickens consume more oxygen (by 0.5 liter) and release more carbon dioxide (by 0.6 liter).

Calculations on the maintenance of ducks and chickens in a closed system with the recirculation of substances can only be tentative, owing to the /118 meagerness of the available zootechnical and physiological data and the absence of suitable experiments.

Less is known about raising ducks than about raising chickens. Methods of feeding chickens by machine are well developed and in wide use. This may be of considerable value in weightlessness or with the use of unfamiliar feed. Problems pertaining to keeping them in coops have largely been solved.

TABLE 8. DAILY FODDER UNIT AND OXYGEN REQUIREMENTS OF THEORETICAL FLOCK OF CHICKENS AND AMOUNT OF CARBON DIOXIDE RELEASED BY THEM.

Age of flock	Average live weight	1st month				2nd month				3rd month			
		No. of chickens	Feed units, g	Oxygen, liter	Carbon dioxide, liter	No. of chickens	Feed units, g	Oxygen, liter	Carbon dioxide, liter	No. of chickens	Feed units, g	Oxygen, liter	Carbon dioxide, liter
1 day old	40	10	100	21.44	17.1	10	100	21.44	17.1	10	100	21.44	17.1
1 month old	450	8	533	204.84	211.41	9	6000	230.44	187.22	9	600	230.44	211.41
70 days old	1030	9	728	439.39	404.17	8	819	390.57	359.26	9	819	439.39	404.17
3 months old	1200	—	—	—	—	1	105	54.46	49.92	—	—	—	—
4 months old	1600	1	117	55.36	51.05	—	—	—	—	1	117	55.36	51.05
5 months old	1900	1	129	69.35	64.06	1	129	69.35	64.06	—	—	—	—
Hens and rooster	2000	8	1208	385.6	329.6	8	1208	385.6	329.6	8	1208	385.6	329.6
Total	—	37	2815	1175.98	1077.39	37	2961	1151.86	1007.86	37	2844	1132.23	1013.13

Table 8 (Continued)

4th month				5th month				6th month			
No. of chickens	Feed units, g	Oxygen, liter	Carbon dioxide, liter	No. of chickens	Feed units, g	Oxygen, liter	Carbon dioxide, liter	No. of chickens	Feed units, g	Oxygen, liter	Carbon dioxide, liter
9	90	19.31	15.39	10	100	21.44	17.1	9	90	19.31	15.39
9	600	230.44	211.41	8	533	204.84	211.41	9	600	230.44	187.92
9	819	439.39	404.17	9	728	439.39	404.17	8	819	390.57	359.26
1	105	54.46	49.92	1	105	54.46	49.92	1	105	54.46	49.92
—	—	—	—	1	117	55.36	51.05	1	117	55.36	51.05
1	129	69.35	64.06	—	—	—	—	1	129	69.35	64.06
8	1208	385.6	329.6	8	1208	385.6	329.6	8	1208	385.6	329.6
37	2951	1198.55	1054.33	37	2791	1161.99	1063.25	37	3068	1201.09	1057.0

TABLE 9. CONTENT OF NUTRIENTS AND CALORIE VALUE OF PRODUCTS OBTAINABLE FROM THEORETICAL FLOCK OF DUCKS AND CHICKENS PER MONTH.

Species of fowl	Amount of product, kg	Proteins, g	Fats, g	Carbohydrates, g	Calorie value, g
			Edible Meat		
Ducks	8.1	1087.3	2073.6	--	13,733
Chickens	5.56	967.4	550.4	--	9,063
			Egg Mass		
Ducks	2.38	251.8	268.5	11.9	3,564
Chickens	2.64	279.8	298.3	13.2	3,960
Ducks	10.48	1339.1	2342.1	11.9	17,297
Chickens	8.2	1247.2	848.7	13.2	13,023

The advantage of ducks is that they can eat a large quantity of green fodder, which will constitute a fairly large portion of the animals' ration on the spacecraft (chlorella, haulm and other inedible (for man) parts of higher plants).

Ducklings mature more quickly than chicks. They gain 40 times their original weight the first two months, while chicks gain over 20 times their original weight.

Thus, each species has its advantages and disadvantages. The final choice of fowl for inclusion in a closed ecological system cannot be made without taking into consideration all other elements in the system.

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PROSPECTS FOR PHARMACOLOGICAL PROTECTION FROM RADIATION INJURY DURING SPACEFLIGHT

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Not too long ago scientists thought that ionizing radiation was not /119 a serious hazard during spaceflight. They conjectured that a large part of the spectrum was occupied by visible light rays, and that the intensity of ultraviolet and roentgen rays was too low to harm the cosmonaut. However, systematic investigations during the past five years have shown this view to be completely untenable. Cosmic radiation, if appropriate protective measures are not devised, may well be one of the major obstacles in the path of man's conquest of space.

Corpuscular radiation in the radiation belts around Earth, solar flare protons and the heavy nuclei of primary radiation pose a genuine threat to the health and even the lives of the crew of a spacecraft, especially on an extended flight. This is now generally acknowledged (refs. 4, 6-9, 12, 13, 16, 20, 21, 25-31, 37, 44, 52, 53, 55, 61, 63 and 65).

Thus, the search for effective pharmacological protective agents is a major scientific and practical endeavor. The utilization of such agents would greatly reduce the weight of the physical (mechanical) protection otherwise required, thereby reducing the overall weight of the vehicle.

The possibility of increasing the resistance of biological objects to /120 the injurious effects of ionizing radiation has now been demonstrated in principle in experiments on animals and by the experience gained in using roentgentherapy for human beings. The details may be found in several monographs and reviews (refs. 23, 24, 37 and 56).

It is pertinent to note here that it was not so long ago that some foreign scientists, especially those supporting the so-called "target" theory, categorically denied that chemical agents could protect the body against radiation. They said that to search for a chemical agent to weaken the effect of radiation was like taking an aspirin tablet in the hope that it would ward off a bullet.

The progress made to date obviously does not mean that the problem of chemical protection against radiation has been completely solved. Only a beginning has been made. The available preparations are far from perfect. The main thing now is to search for agents that can be used in medical practice. Everyone

knows that not every preparation which provides satisfactory protection in animal experiments can be used by human beings because of side effects, toxicity or other negative features. For example, although potassium cyanide is effective in animal experiments, it is highly toxic and therefore has no practical value.

Chemical protection against radiation during spaceflight has been given comparatively little experimental attention. Drugs like mercamine, mercamine disulfide, AET, serotonin, 5-methoxytryptamine and some others are known to have a good protective effect in animals subjected to X or gamma rays at a comparatively high dose rate. Animals received a lethal dose in a comparatively short period of time (5-40 minutes).

Some of the agents (mercamine hydrochloride, mercamine salicylate, mercamine disulfide, AET) have also been quite effective in human beings (refs. 1, 5, 17-19, 22, 32, 33, 38-42, 45, 46, 49, 51, 54, 62 and 66). However, they were only irradiated locally. During spaceflight the cosmonauts and other biological objects that form part of the closed ecological system of a spacecraft will be continuously exposed to radiation, generally at a very low dose rate.

We do not know whether the available radioprotective agents will be /121
effective under such irradiation conditions. For example, cystamine, distaphen and isotiphen are less effective with general fractional irradiation than after a single exposure. Moreover, they were tested on animals exposed to rare ionizing radiation. However, it is known from the literature that the protective action of some agents decreases significantly or is even absent if the biological objects are exposed to dense-ionizing radiation (refs. 48, 56 and 57). Recent experiments on mice (refs. 35 and 36) have shown that some preparations of the aminothiol series (cystamine, cysteamine, serotonin, AET, etc.) have a marked protective effect in animals exposed to protons with an energy of 660 and 120 Mev.

These data indicate that the aminothiol preparations have a protective effect against roentgen and gamma rays as well as high-energy protons. This is important, because protons are one of the main components of cosmic radiation. Unfortunately, for a number of reasons we were unable to carry out experiments to determine the protective effect of these against proton irradiation at a low dose rate. Of course, cosmic radiation consists of more than protons. During spaceflight the cosmonauts and other biological objects will be exposed to mixed radiation with very high energies, which we are still unable to reproduce in the laboratory.

We must stress that during spaceflight all biological objects are exposed to many factors: ionizing radiation, acceleration, vibration, weightlessness, noise, change in barometric pressure and composition of gases, etc., which affect the organism in the greatest variety of combinations and sequences. It is self-evident that to form correct judgments about an expected biological action requires a knowledge of the sequence, intensity and duration of action of the individual factors, including the different kinds of ionizing radiation encountered in passing through the various regions of outer space.

Irradiation of the cosmonauts will be distributed unevenly in time as a result of the radiological heterogeneity of space. Moreover, the nature of the individual response to radiation combined with other factors will be different. For example, the reaction to going into orbit is obviously different from that to orbital flight or landing. Unfortunately, there have been few flight /122 or even laboratory experiments on the combined effects of radiation and other factors. We recently obtained some experimental data on mice, rats and guinea pigs, which showed that vibration and acceleration applied once 4 or 24 hours before irradiation with protons or gamma rays in injurious doses either failed to influence or even slightly mitigated the course of radiation sickness. But if vibration or acceleration was applied after irradiation, either factor significantly aggravated the course of radiation sickness in rats.

Solution of the problems involved in the combined effects of flight factors requires a comprehensive evaluation of all the conditions obtained in the cabin of a spacecraft: nutrition, water supply, air regeneration, work and rest routines, emotional and mental states, sanitary and hygienic conditions. The importance of these conditions and individual peculiarities is well known to present-day radiobiology.

It is, of course, impossible to reproduce the effects of all these factors in laboratory experiments. However, the influence of some of them can be simulated in experiments on animals and in laboratory experiments, the results of which can later be refined in flight experiments.

Not all radioprotective agents, effective under ordinary ground conditions, can be recommended for use when cosmic radiation is combined with other flight factors. Our experiments on mice and dogs, although few in number, have shown that some agents sharply decrease resistance to vibration and acceleration. Some agents affect the tolerance of these factors differently, depending on the time they are administered.[1]

These investigations have just begun, and there are, of course, few facts as yet. Nevertheless, limited though the experimental findings may be, they suggest that recommendations for the use of this or that agent during spaceflight should be treated with great caution. Such agents must satisfy /123 rigid requirements, which we shall not go into here because we have discussed them in detail elsewhere (ref. 28).

Higher and lower plants, microorganisms and other representatives of the animal and plant worlds will routinely be carried aboard space vehicles to form part of the ecological system. It is possible that under the influence of cosmic radiation and other flight factors genetic and cytological changes may arise which, in the absence of suitable protective measures, could disrupt the balance of the ecological system and thereby create conditions incompatible with the vital activity of the crew. This is the reason why we must now study the possibility of developing resistant ecological systems. Adequate attention must also be paid to finding radioprotective agents for these biological objects. The demands made on these preparations will obviously be somewhat different from those made on preparations intended for man.

[1]These findings will be published after additional experiments.

In the light of the foregoing, we can see that the search for effective radioprotective agents for the crew and the various biological objects is an urgent and highly complex problem, one that has not been sufficiently studied. It will not be solved without the participation of specialists in a variety of fields (pharmacology, chemistry, radiobiology, microbiology, genetics, etc.). Efforts must be made both to improve existing agents and to synthesize new ones.

We wish to lay special stress on the need for laboratory experiments to study chemical agents in models as close as possible to actual flight conditions, at least those involving the radiation factor. What we have particularly in mind is the dose rate. It will apparently be procedurally sound to test compounds with presumptive protective action against prolonged, continuous irradiation at a low dose rate.

Agents with good radioprotective properties must be tested in experiments involving radiation combined at least with those flight factors which can be reproduced in the laboratory (e.g., vibration, acceleration).

Any drug recommended as a means of individual radioprotection for the crew /124 members must have the fullest pharmacological characteristics. Otherwise, for obvious reasons, there can be no question of its practical use.

We should like to state, in conclusion, that, despite the complexity of the problem, we are certain that it will be solved.

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BIOLOGICAL EVALUATION OF RADIATION CONDITIONS ON AN EARTH-MOON TRAJECTORY[1]

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Possibilities for the investigation and mastery of outer space by /127
man, some aspects of which K. E. Tsiolkovskiy had originally formulated, imply, in particular, flights by human beings to other planets of the solar system.

The Moon will obviously be the first celestial body to be reached by man in his search to unfathom the mysteries of the Universe. This is only natural because the investigation and biological evaluation of the physical and especially radiation conditions that man will encounter during flight to the Moon are of great scientific interest.

Numerous studies have already been made in this area; data have been obtained on different parameters and properties of certain physical factors of periterrestrial space, thus making it possible to determine, to some extent, the dimensions of anticipated hazards and man's possibilities for such a flight. We have in mind, first of all, the investigation of the composition, the energy spectrum, the spatial and temporal distributions of cosmic radiation and research on the biological effectiveness of various forms of ionizing radiation.

Some success has been achieved in studying the effect of weightlessness on the human organism. V. F. Bykovskiy's flight, as well as the flights of other cosmonauts, gives us reason to hope that man will be able to achieve flights in excess of 5 days, while tolerating weightlessness and maintaining his working capability (refs. 1-3).

The literature contains some interesting data on technical means for /128
realizing the idea of a flight by man to the Moon (refs. 4 and 5). However, it is perfectly clear that the problem of technical and medicobiological arrangements is far from being completely solved. Even the projected time bracket of 1967-1970 (ref. 6) for making such a flight should be considered as most tentative. No doubt it will depend to a large extent on the results of further study of physical conditions in interplanetary space, on the results of surveying the Moon itself and on solving a number of medicobiological problems for making such a flight safe.

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[1] This report was read at the XV International Astronautic Congress held at Warsaw October 7-12, 1964.

The present article attempts to examine the radiation picture in periterrestrial space and to evaluate the danger from ionizing radiation which may be encountered by man in his flight trajectory from Earth to the Moon.

Prior to the discovery of radiation belts around Earth (refs. 7 and 8), ionizing radiation was not considered a factor materially affecting flight safety (ref. 9).

With the discovery of radiation, and especially irradiation zones accompanying chromospheric flares on the Sun, cosmic radiation began to be considered as one of the main obstacles to man's penetration of the cosmos (refs. 10-12).

As we know, cosmic radiation is composed of galactic rays (primary cosmic irradiation), ionizing irradiation of periterrestrial radiation zones and radiation created during chromospheric flares on the Sun. A special place is occupied by irradiation from artificial radiation zones formed as the result of nuclear bomb explosions in space.

In order to evaluate these forms of irradiation from the point of view of radiation hazard, we must examine the composition of each form of irradiation, its energy spectrum, the interaction of charged particles with matter and the biological dose.

Primary Cosmic Rays (PCR)

These rays present a special shower of charged particles, primarily protons, arriving practically isotropically from outer space. At the present time it is customary to assume that the shower of PCR consists approximately of 85 percent protons, 13-14 percent α particles, and 1-2 percent particles with $z \geq 3$ charge.

It is believed that all primary particles lack all their orbital electrons, i.e., they are the nuclei of different elements. The relative distribution of these nuclei among PCR is similar to cosmic distribution of the respective elements, but differs from the latter by a profusion of heavy nuclei. This is especially characteristic of the composition of irradiations emanating long distances away from Earth. The energy of PCR particles embraces a very wide range (from 20-40 to 10-12 MeV/nucleons).

Despite the large number of experiments for determining the charge spectrum of primary particles, no precise form has as yet been established. This /129 is due to the low intensity and difficulty of identifying nuclei that are heavier than helium. Data obtained from the launching of Soviet space rockets indicate that the intensity of primary cosmic rays in interplanetary space fluctuates on the basis of an 11 year solar cycle from 2 (maximum activity) to 4.5 part/cm^{-2}/sec^{-1} (ref. 13).

Let us attempt to estimate a dose of PCR which a cosmonaut would receive when beyond the limits of Earth's magnetic field. The estimated daily dose

TABLE 1. DAILY DOSE GENERATED BY PCR IN OUTER SPACE FOR DIFFERENT GROUPS OF PARTICLES.

Particles or groups of nuclei	Shower, part·cm^{-2}/sec^{-1}	Daily dose, mrad	Daily dose, mrem
Protons	1.8	4.9	5.0
α particles	0.4	5.5	5.5
L ($3 \leq z \leq 5$)	$1 \cdot 10^{-2}$	0.4	0.5
M ($6 \leq z \leq 10$)	$2 \cdot 10^{-2}$	5.5	33.0
H ($z > 10$)	$1 \cdot 10^{-2}$	5.2	96.0
Total	2.24	21.5	140.0

figures due to PCR in outer space are presented in table 1, according to different groups of particles.

Taking into account the results of studies conducted on space rockets (ref. 13), we computed the intensity of PCR to be 2.24 part/cm^{-2}/sec^{-1}. The relative biological effectiveness (RBE) coefficient for different particles was determined on the basis of results of the graphic relation RBE to linear losses of energies (LLE), cited in reference 14.

If we assume that the composition of PCR is relatively constant, the daily dose would fluctuate between 125 and 270 mrem, depending on solar activity, which is 7-16 times the permissible daily dose for persons constantly working with sources of ionizing radiations (ref. 15). The protective shell of a capsule equal in thickness to 1-2 g/cm^2 would hardly affect this magnitude.

We pointed out that cosmic rays have electrons amounting to ~ 1 percent and an insignificant shower of gamma rays not perceptibly contributing to ionization. In considering the problem of radiation danger from PCR, ionization stemming from electrons and gamma rays may be ignored.

As is known, Earth's magnetic field deflects a considerable number of PCR particles. For this reason, the integral PCR dose on orbits flown by /130 Soviet and American cosmonauts is almost half the dose found in outer space. Table 2 gives the total doses received by Soviet cosmonauts in flights on Vostok spacecraft.

The table shows that the mean daily dose obtained experimentally corresponds (within measuring tolerances) to the rated dose. In this connection, 85-90 percent of the integral dose obtained during such flights is attributed to primary cosmic radiation, its main component (ref. 10).

Experiments conducted on recoverable sputnik capsules contributed to a fairly complete evaluation within the framework of the radiobiological picture of the dose of cosmic radiation obtainable at elevations of 180-320 km. For this purpose, various biological specimens were used, representing various

TABLE 2

Cosmonaut	Duration of flight, hr	Type of dosimeter					
		ILK (3.2 mm AL)		IK (0.6 mm)		R-3	
		Dose	mrad/ daily	Dose	mrad/ daily	Dose	mrad/ daily
Yu. A. Gagarin	1.5	0.5	8.4	-	-	-	-
G. S. Titov	25	13 ± 2	12 ± 2	-	-	-	-
A. G. Nikolayev	94	64 ± 1	16 ± 1	58 ± 7	15 ± 2	43 ± 1	11 ± 1
P. R. Popovich	71	48 ± 1	16 ± 1	51 ± 7	17 ± 2	32 ± 1	11 ± 1
V. F. Bykovskiy	119	75 ● 2	15 ± 1	81 ± 6	16 ± 1	50 ± 1	10 ± 1
V. V. Tereshkova	71	48 ± 1	16 ± 1	42 ± 2	14 ± 1	30 ± 1	10 ± 1

stages of organization, from bacteria and microspores to dogs. Special methods of study were employed for this purpose (refs. 16-19).

Analysis of the results disclosed only a few modifications for lysogenic bacteria, a number of plants and Drosophila. These modifications, which primarily were concentrated in the genetics apparatus of the specimens, may be attributed to the action of cosmic radiation.

For example, during the flight of Vostok 5, lasting almost 5 days, the total radiation dose registered 80 mrad on the physicochemical dosimeters and 50 mrad on the gas discharge meter. If we take 10 as the RBE coefficient for the heavy component of PCR, then the rem would be approximately 450-750 mrem. A total dose of this size can be determined only with the assistance of specimens that are extremely sensitive to irradiation and corresponding tests. Such a scheme explains the recording of modifications, for example, in lysogenic bacteria and the absence of radiobiological effects in organisms less sen- /131 sitive to radiation.

During the flight of a human being in free outer space, the integral PCR dose doubles, increasing during a 15 day flight, for example, from 1.8-4 rem. As a radiation hazard, such a dose during the course of a flight to the Moon does not actually exceed permissible levels of irradiation established for cosmonauts and can hardly exert any significant effect on the health of a human being.

However, in view of the biophysical peculiarities of the action of heavy nuclei connected with the ability of particles to produce great density of ionization in the terminal part of a run ("impact" phenomenon), we would like to emphasize the thesis that the lodging of heavy nuclei in the hypothalamic region of the brain, the crystalline lens, the retina, etc. could incapacitate a large group of cells and thus disrupt, for example, thermoregulation, causing cataracts and microscotomas, etc.

The "impact" phenomenon constitutes a new type of radiobiological action, the study of which has just begun. At the present time, more or less convincing facts are available concerning the action of the heavy component. These were obtained in both flight and laboratory experiments on small biological specimens and individual cells. But there is a total absence of experimental data on the possible effect of local affections of certain organs and centers on the whole organism. On the basis of certain theoretical estimates (refs. 20-22) and biological experiments conducted on Soviet and American flight craft (refs. 18 and 23-25), it is assumed that the probability of such local affection of individual groups of cells, should it exist, would be small and of no practical importance in flights lasting up to several weeks.

Ionizing Irradiations of Earth's Radiation Belts

Radiation of the inner belt consists of protons exceeding 40 MeV in energy. Their spectrum changes according to the law $N/ > \varepsilon = K\varepsilon^{\gamma}$ with the index $\gamma = 1.84$ and electrons with an energy E_e 20 keV. Maximum intensity according to different data is $2 \cdot 10^4$-$2 \cdot 10^8$ part/cm^2/sec for protons. The width of the belt with the greater intensity of irradiation is approximately 4,000-5,000 km. Upon leaving Earth, a spacecraft would remain in this belt for about 15 min. The average size of the dose created by protons during that segment of the orbit which passes through the inner belt would be about 5 rem/hr within a spacecraft protected by 1 g/cm^2. Electrons of the inner radiation belt would be completely intercepted by such protection, while the size of the dose, because of bremsstrahlung, would equal approximately 0.1 rad/hr.

Radiation of the outer belt consists fundamentally of electrons with E_e from 20 keV to several MeV. The intensity of electrons with an energy of /132 $E_e > 40$ keV in the center of the belt is equal to 10^8 part/cm^2/sec. Radiation from this belt should not materially affect the total dose, even though it would take about two hours for a spacecraft to pass through it. This contribution in relation to a protection of 1 g/cm^2 would be equal to 0.2-0.3 rad because of bremsstrahlung.

Electrons found by Soviet investigators outside the limits of the outer belt (ref. 26) are completely kept out by this protection and do not affect the integral dose because of their weak energy.

A cosmonaut, therefore, on board a spacecraft with a protection of 1 g/cm^2 would receive a total dose of the order of 2.5-3.5 rem. Such a dose, just like the PCR dose, would not constitute a threat to the cosmonaut's health.

In the artificial zone, radiation intensity, spatial distribution of radiations and the life of injected particles depend on a large number of factors, which at the present time cannot be computed theoretically. Predictions made on the basis of individual experimental facts have not been corroborated in many cases.

The measurement of doses in the center of the artificial belt formed as the result of a nuclear explosion set off by the USA on July 9, 1962 indicated the presence of high levels of radiation. With a protection of 4.5 g/cm^2 the size of the radiation dose was 3 rad/hr two months after the explosion and 35 rad/hr four months later. With a protection of 0.4 g/cm^2, the size of the radiation dose was 2,000 rad/hr two months after the explosion and 30,000 rad/hr 4 months after. Measurements of these zones made one year after the explosion showed the perseverance of a high radiation level; the size of the dose with a protection of 3 g/cm^2 was 17 rad/hr (ref. 27).

The nuclear explosion of July 9, 1962 thus resulted in the formation of a rather large zone, in which the electrons create approximately the same hazard that protons do in the inner belt. Future dynamics of the artificial zone are hard to predict. For this reason the radiation hazard for a specific flight can be determined only after direct measurements of the radiation levels in the given region. If a flight along a trajectory for circling the Moon is forced to pass through such conditions, a cosmonaut in passing through the artificial zone protected by 1-2 g/cm^2 will receive a dose of 2-3 rem from bremsstrahlung.

Radiation Occurring at the Time of Chromospheric Flares on the Sun

Such radiation is composed of approximately 90 percent protons and 10 percent α particles. Heavy nuclei up to Z = 18 were observed during some flares (ref. 28). /133

Emissions of solar protons may be divided arbitrarily on the basis of intensity and energy spectrum into three groups: high-, medium-, and low-energy.

The group of high-energy flares includes emissions, the secondary radiation of which can be observed on Earth at sea level. The energy of protons from such flares reaches 20 BeV, the duration, several dozen hours. The intensity is relatively light. Beginning with 1942, such flares included those which took place on March 7 and 28, 1942, July 25, 1946, November 19, 1946, and February 23, 1956. The last flare was maximal. Characteristically, these flares occurred either on the wane or rise of solar activity. They take place on the average every 4-5 years.

Examples of medium-energy flares include those which took place on July 10 and 16, 1959 and November 12, 1960. The energy of protons from such flares varies from one to several BeV. They occur 2-4 times during a year of high solar activity. The third group includes flares of the type that took place on Aug. 22, 1958 and May 10 and Sept. 14, 1959. The energy of protons here was several hundred MeV. The frequency of such flares was 10-12 times a year.

In regard to optic brightness, solar flares are divided into 7 classes (1, 1$^+$, 2, 2$^+$, 3, 3$^+$, 4). Generation and emission of radiationally hazardous protons from solar flares are usually accompanied by optic flares of class 3 or 3$^+$.

In order to determine the degree of radiation hazard from protons of solar flares for the purpose of designing protection measures against them, it is essential to know the flux and energy spectrum for the entire extent of the flare, particularly for the phase of maximum intensity of the flux. This is required because the fundamental contribution to the dose occurs during this period. It is also necessary to know the direction of movement of the main masses of charged particles.

At the present time computations of doses exist, made by different authors, and calculations of the required protection against proton radiation of the different classes of flares. Estimated dose sizes and recommendations for physical protection vary widely, depending on the nature of initial data used in the computations, conditions of flight, etc. However, all computations, regardless of how they may have been achieved, clearly indicate the great danger from protons of solar flares, especially in flights by man outside the limits of Earth's magnetic field.

In order to prevent an integral dose from exceeding 100 rem, it is necessary to use a protection of 13 g/cm^2 for a flare of the class of February 1956, 15 g/cm^2 for a flare like that of May 1959, and 2 g/cm^2 for a flare like /134 that of August 1958. A decrease in the radiation dose to the permissible threshold level of 25 rem (ref. 11) would require a protection from these flares to be 32.25 and 2.8 g/cm^2, respectively. It is perfectly obvious that the fulfillment of such demands presents major and hitherto unresolved technical difficulties (ref. 36).

A cosmonaut in interplanetary space with a protection of about 3 g/cm^2, passing through a radiationally hazardous solar flare, may receive a dose anywhere from several dozen to several hundred rad. The question naturally arises as to how such doses should be evaluated biologically. We know that a single irradiation with a dose of about 25 rem on Earth would produce specific changes with respect, for example, to the blood and central nervous system, while a dose of approximately 100 rem would bring on initial reactions of nausea, heightened fatigue and other pathologic symptoms, accompanied by lowered working capability. A single irradiation with a dose of 200 rem in 50 percent of cases causes a typical acute radiation sickness with nausea, vomiting, vertigo and loss of working capability. Fatalities are found following the action of irradiation with a dose of approximately 300 rem, but they may occur with smaller doses.

Flight conditions seemingly modify the organism's reaction to ionizing radiation. However, an evaluation of such factors during the course and outcome of radiation sickness is fraught with obstacles at the present time.

The radiobiological effect is due to many reasons--size of absorbed integral dose, kind of radiation (density of ionization), duration of irradiation (strength of dose), extent of irradiation of organism (whether whole-body or partial), the functional condition of the organism and its radiation resistance.

Two factors probably can decrease the effect of radiation from solar flare protons--relative time diffusion of action (small size of dose) and use in the capsule of different screens for preventing total irradiation. At the same time, certain flight conditions (emotional tension, modified regimen, decreased motor activity, modified gaseous composition, etc.) decrease resistance of the organism to radiation and enhance the effect of irradiation.

A considerable lack of clarity exists with respect to the effect of weightlessness on reaction to irradiation. Initial experiments in this area give reason to believe that the effect of cosmic radiation may be cumulative with respect to the action of prolonged weightlessness (ref. 29).

In evaluating the course and outcome of radiation sickness, we should keep in mind not only factors that are operative during a flight, but also factors that exert an influence on the organism during the descent of a spacecraft. We are referring particularly to the effect of acceleration on the cardiovascular system, the resistance of which declines considerably under the /135 influence of ionizing radiation.

The relative biological effectiveness (RBE) of solar protons may be determined at the present time only indirectly on the basis of calculations and results of laboratory experiments conducted on proton accelerators.

Analyzing the experimental results obtained by a number of authors (refs. 30-34), we conclude that the coefficient of RBE for protons of solar flares, if we take into account the radiation spectrum as a whole, should be more than 1 ($\sim$ 1.5). In estimating the integral dose received by a cosmonaut from a flare, we must remember that under certain conditions a considerable contribution to this dose may be made by secondary neutrons, for which the RBE coefficient would not be less than 2.

Summarizing, we can say that the effect of radiation during flights would be complicated by the action of a number of factors (coefficient of intensification being not less than 1.5). This consideration must be taken into account when determining the limits of permissible radiation levels for cosmonauts in the development of protective measures.

It is quite obvious that protons from solar flares are one of the main sources of acute overirradiation of a cosmonaut when he is beyond the limits of Earth's magnetic field.

What is the probability of a spacecraft encountering a radiation hazard and a cosmonaut receiving in consequence an overdose of irradiation during the course of a trajectory flight around the Moon? This depends on the mean probability of a flare taking place and on the duration of flight. Such a hazard is relatively high for a flight lasting one week, being 16 percent for a flare of the type of August 22, 1958, 5.8 percent for the flare of May 10, 1959, and 0.3 percent for the flare of February 23, 1956 (ref. 35). This danger would increase during a period of heightened solar activity and with lengthening of flight time.

We have thus briefly examined the physical characteristics of the basic types of cosmic radiation and determined biological dose sizes (essentially the upper limits) for each kind of radiation. According to our findings, the integral radiation dose from PCR, the radiation of natural and artificial periterrestrial belts, with a protection of 1-2 g/cm^2, should not exceed 10 rem for a two week flight along a trajectory circumnavigating the Moon. For an emergency return from an altitude of about 75,000 km along the most disadvantageous trajectory, the maximal permissible dose would be about 20 rem (ref. 36). Consequently, a protection of 1-2 g/cm^2 would be adequate for the members of the crew, provided that the flight takes place during a period of quiet solar activity.

The protons of solar flares are a real threat to the health and lives /136 of cosmonauts. With this in mind, it would be desirable to increase the physical protection to 3 g/cm^2. For a flare of the type of August 22, 1958, this would decrease the integral dose to a permissible level (refs. 11 and 15). The problem of physical protection from protons generated during flares of the type of July 10, 1959 and February 23, 1956 is extremely complex and at the present time evidently unsolvable.

In considering problems of physical protection of cosmonauts from the action of ionizing radiation, we must keep in mind two factors, pointed out in a number of investigations (refs. 36-39). First, in the design of spacecraft, advantage should be taken of stores of fuel, water, food, etc. for protection purposes. Secondly, in determining the optimal dimensions of protection, we must take into account the high energies and varied composition of PCR and solar radiation. In a number of instances it would not be practicable to increase the thickness of the protective layer, because such a measure could result in increasing rather than decreasing the integral dose (ref. 40). The physical properties of materials, their shape, etc., are very important in determining protective measures.

Aside from the use of physical protection, what other measures could be employed to decrease the danger from proton radiation during solar flares? First, existent forecasting methods can predict with an accuracy of 75 percent the appearance of flares 2-3 days in advance. Such a period is short. Consequently, forecasting should be given intensive study, together with the development of suitable apparatus set up not only at terrestrial stations, but also in spacecraft.

Secondly, the resistance of the organism to the action of protons from solar flares can be considerably increased by use of pharmacochemical preparations (refs. 11, 12 and 41). Successful research in this area promises that pharmacochemical protection of cosmonauts from ionizing radiation will become one of the main links in the system of measures for providing radiation protection in spaceflights.

In conclusion, we point out that progress in space exploration by man is connected with the continued accumulation of information on physical parameters

and properties of outer space and biological evaluation by experimental flight. A great deal of importance must be attached to the biological indications of spaceflights.

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ANTIRADIATION PROTECTION IN CONNECTION WITH THE PROBLEM OF THE RELATIVE BIOLOGICAL EFFECTIVENESS OF RADIATIONS WITH LOW SPECIFIC IONIZATIONS

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Until 1961 there was no information avialable on the use of protec- /139
tive agents against high-energy radiation. Most of the investigations involved X- or gamma rays. It was shown that in contrast to the action of the latter, the protective effect of various agents is weak or nonexistent (ref. 108) against corpuscular radiation, which is characterized by high linear energy losses (LEL). In recent years interest has grown considerably in the biological effects of high-energy radiations, chiefly protons with an energy of the order of hundreds of MeV. This has come about largely because of the vigorous efforts made to conquer space, the radiation danger of which stems to a great extent from the high-energy protons that constitute the main component of the Earth's inner radiation belt and galactic and solar radiations (refs. 4 and 85). Unlike the corpuscular radiation used in most of the earlier radiobiological experiments, the LEL of high-energy protons approaches the corresponding values of hard electromagnetic radiations.

The problem of ensuring the radiation safety of spaceflight cannot be solved without experimental data on the relative biological effectiveness (RBE) and degree of protection afforded by various agents in relation to the nature of the radiations with respect to the LEL (refs. 4 and 51). This is particularly applicable to the projected interplanetary flights and to the possibility of coping with emergency situations that may arise during orbital or lunar flights.

Such studies will also add to our knowledge of the mechanisms responsible for the radiobiological effects (ref. 69).

1. RBE of Hard Radiations of Electromagnetic Nature

The problem of the RBE of ionizing radiation, as noted by M. P. /140
Domshlak and N. T. Darenskaya (ref. 20) was correctly formulated for the first time by Gray in the 1940s (refs. 90 and 91).

Since then it has been thoroughly studied in many laboratories throughout the world. Attention has been centered on the various aspects of the biological action of the gamma radiation of radioactive isotopes, X-rays and electrons in a wide range of energies, and thermal and rapid neutrons. Investigations are

TABLE 1. RBE OF RARE TYPES OF ELECTROMAGNETIC RADIATION (LEL IN KeV/μ OF RANGE).

Recorded effect	Standard radiation		Comparable radiation		RBE	References
	Type	LEL	Type	LEL		
Destruction of Escherichia coli cells	X-rays 250 kV	2.6	X-rays 1,000 kV	0.4	0.91	(96)
Same	X-rays 180 kV	3.0	X-rays 31 MeV	0.2	1.0	(102)
Same, strain B/2	X-rays 180 kV	3.0	X-rays 22.5 MeV	0.2	0.93	(101)
Destruction of yeast cells	X-rays 200 kV	2.9	γ rays Co^{60}	0.3	0.85	(117)
Destruction of yeast cells	X-rays 200 kV	2.9	X-rays 22 MeV	0.2	0.85	(27)
Same, haploid strain	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.76	(27)
Same, diploid strain	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.84	(27)
Destruction of Vicia faba root cells	X-rays 185 kV	3.0	γ rays Ra^{226}	0.3	0.72	(83)
Chromosome exchanges in Tradescantia microspores	X-rays 220 kV	2.8	X-rays 30 MeV	0.2	0.8	(83)
Same	γ rays Co^{60}	0.3	X-rays 30 MeV	0.2	0.9	(83)
Chromosome aberrations in Tradescantia microspores	X-rays 60 kV	4.8	γ rays Co^{60}	0.3	0.67	(83)
Same	X-rays 200 kV	2.9	γ rays Co^{60}	0.3	0.5	(83)
Same	X-rays 220 kV	2.8	X-rays 30 MeV	0.2	0.7	(83)
Cell growth in a culture	X-rays 200 kV	2.9	γ rays Co^{60}	0.3	0.86	(94)

TABLE 1 (Continued)

Recorded effect	Standard radiation		Comparable radiation		RBE	References
	Type	LEL	Type	LEL		
Cell growth in a culture	X-rays 200 kV	2.9	X-rays 22 MeV	0.2	0.86	(94)
Chromosome aberrations in a culture of human cells in different phases of the cell cycle	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.17-0.86	(22)
Recessive sex-linked mutations in Drosophila melanogaster	X-rays 180 kV	3.0	X-rays 31 MeV	0.2	0.6	(88)
Same	X-rays 200 kV	2.9	X-rays 20 MeV	0.2	0.7	(103)
Same	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	0.9	(86)
Dominant lethals in Drosophila melanogaster	X-rays 180 kV	3.0	X-rays 22 MeV	0.2	0.8	(118)
Same	X-rays 300 kV	2.4	X-rays 4 MeV	0.3	0.9	(77)
$LD_{50/30}$ in mice	X-rays 250 kV	2.6	X-rays 1,000 kV		0.83-0.85	(87)
Same	X-rays 300 kV	2.4	X-rays 4 MeV	0.3	0.74	(111)
Same	X-rays 250 kV	2.6	γ rays Ra^{226}	0.3	0.75	(119)
Same	X-rays 1,000 kV	0.4	γ rays Ra^{226}	0.3	1.0	(119)
Same	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	0.75	(119)
Same	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	0.77	(119)
Same	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	0.79	(95)

TABLE 1 (Continued)

Recorded effect	Standard radiation		Comparable radiation		RBE	References
	Type	LEL	Type	LEL		
$LD_{50/30}$ in mice	X-rays 250 kV	2.6	γ rays Ra^{226}	0.3	0.7	(119)
Same	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.74	Personal data
$LD_{100/30}$ in mice	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.8	Personal data
$LD_{100/4}$ in mice	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.85-1.0	Personal data
$LD_{50/30}$ in rats	X-rays 400 kV	2.0	X-rays 22 MeV	0.2	0.75	(106)
Same	X-rays 200 kV	2.9	X-rays 22 MeV	0.2	0.85	(115)
Same	X-rays 200 kV	2.9	γ rays Co^{60}	0.3	0.86	(115)
Same	γ rays Co^{60}	0.3	X-rays 22 MeV	0.2	1.0	(115)
$LD_{50/30}$ in rats	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	1.0	(21)
$LD_{100/30}$ in rats	X-rays 180 kV	2.0	γ rays Co^{60}	0.3	1.13	Personal data
$LD_{100/30}$ in rabbits	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	1.5-1.7	(21)
$LD_{100/30}$ in dogs	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	1.3-1.6	(21)
$LD_{50/30}$ in dogs	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	1.5	(21)
Atrophy of spleen in mice	X-rays 250 kV	2.6	γ rays 4 MeV	0.3	0.54	(119)
Same	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	1.0	(119)

TABLE 1 (Continued)

Recorded effect	Standard radiation		Comparable radiation		RBE	References
	Type	LEL	Type	LEL		
Atrophy of spleen in mice	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	1.0	(95)
Atrophy of thymus in mice	X-rays 300 kV	2.4	X-rays 4 MeV	0.3	0.92	(111)
Same	X-rays 250 kV	2.6	γ rays Co^{60}	0.3	1.0	(119)
Same	X-rays 250 kV	2.6	γ rays 4 MeV	0.3	0.8	(119)
Atrophy of testes in mice	X-rays 250 kV	2.6	X-rays 1,000 kV	0.4	0.83-0.85	(87)
Same	X-rays 300 kV	2.4	X-rays 4 MeV	0.3	0.79	(111)
Same	X-rays 260 kV	2.6	γ rays Co^{60}	0.3	0.9	(119)
Same	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.73	(47)
Incorporation of Fe^{59} in rat erythrocytes	X-rays 200 kV	2.9	γ rays Co^{60}	0.3	0.85	(116)
Same	X-rays 200 kV	2.9	X-rays 22 MeV	0.2	0.84	(116)
Same	X-rays 1,000 kV	0.4	γ rays Co^{60}	0.3	0.99	(116)
Same	γ rays Ra^{226}	0.3	γ rays 4 MeV	0.3	0.6	(119)
Chromosome aberrations in mouse bone marrow cells	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.7	Personal data
Chromosome aberrations in rat liver cells	X-rays 180 kV	3.0	γ rays Co^{60}	0.3	0.7	(44)

under way on the most varied levels of biological organization from bacteria to man, using a great variety of indices.

A detailed examination of the whole problem of RBE is beyond the scope of this survey, and interested readers are referred to the pertinent literature (refs. 20, 82, 83, 89, 97 and 119). We shall focus here on the RBE of radiations with low specific ionizations, the data having been obtained in experiments on microorganisms, plant and animal cells, insects and mammals.

Study of RBE and evaluation of the literature is complicated by the need of a precise differential approach to the analysis of each index, with due regard for the relationship between the RBE coefficient and a host of factors. The latter include the object under study, the effect recorded, size of the dose, dose rate or fractionation conditions, type of radiation source, form of beam and dose field, LEL in the entire depth of the object, direct and remote effects, reversible and irreversible injuries and somatic and genetic effects (ref. 82).

It will be remembered that realization of the effect of radiation is related primarily to the exchange of energy resulting from ionization. Consequently, the differences observed in the reaction to the various kinds of ionizing radiation are attributable not to certain characteristics of the latter, but to the distribution of the radiation in space and time, independent of its physical nature (ref. 20).

Table 1 presents the most characteristic RBE values obtained from published sources and our own experiments in comparison with the LEL values. For convenience of analysis, we took as standard radiation the denser ionizing radiation and compared its effect with that of the radiation under study.

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Analysis of the results of the experiments as a whole reveals a general pattern of decrease in RBE with decrease in LEL. The RBE of high-energy gamma and X-rays ranges in different objects and for the various indices from 0.5-0.9, with 1.0 taken as the effect of standard X-radiation (180-250 kV).

This range of fluctuation of the RBE coefficient is due to the circumstances mentioned above, the determining ones being the nature of the effect recorded and the radiation conditions. The clearest RBE-LEL relationship is seen in the experiments performed on mice to determine the survival time of the animals after irradiation or the dynamics of change in weight of the testes. On the other hand, the degree of atrophy of the thymus or spleen cor- /144
relates poorly with the LEL value, apparently because of the marked variability normally characteristic of these indices.

In studying RBE at the cellular level, the most precise data were obtained from analyzing chromosome injuries. For most biological objects, the coefficient of RBE is, in general, approximately proportional to the cube root of the LEL of the type of radiation under study (ref. 60).

However, analysis of the determinations of the RBE in larger animals (beginning with rats) showed that the value of the RBE coefficient is affected

TABLE 2. RELATIONSHIP BETWEEN RBE, RADIATION HARDNESS AND SPECIES OF ANIMAL.

Animal	$MLD_{100/30}$		RBE of Co^{60} γ rays
	X-rays, 180 kV	γ rays, Co^{60}	
Mice	700	850	0.82
Rats	850	750	1.13

by the nature of the distribution of [illegible]ant energy in the body mass. This is due to the fact that standard X-radiation is not as effective as gamma radiation, which is responsible for raising the RBE of the latter above 1.0. These circumstances must be taken into account when analyzing the experiments involving unilateral X-radiation of rats or bilateral radiation of rabbits and especially dogs from a RUM apparatus.

Table 2, which includes our own data based on a long-term experiment, may give rise to the incorrect conclusion that the radiosensitivity of mice and rats changes with the type of radiation. The reason for this phenomenon is found in the radiation conditions. With unilateral X-radiation of 180 kV, the energy distribution in the body of mice is relatively more uniform than in the body of rats, in whose radiosensitive organs (especially in bone marrow) the tissue dose is significantly less than in the surrounding tissues (ref. 114). With gamma irradiation, due to the greater penetrating power of gamma rays, the unevenness of energy distribution is considerably leveled. The same results can be obtained in a similar fashion by circular X-radiation of rats on a multitubular apparatus. The energy distribution of X-radiation in the larger animals (rabbits, monkeys, dogs) will apparently always be uneven regardless of the design of the irradiating unit. /145

2. RBE of Heavy High-Energy Particles with a Low LEL

Investigations of the biological effects of high-energy protons (660 MeV) were undertaken for the first time in the Soviet Union at the Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Sciences, in 1959. By 1961 the first experimental data were obtained on the RBE of protons of 660 MeV (refs. 2, 43 and 55). This information made it possible to broaden comparative studies on radiation protection and to describe in detail the radiobiological effects of high-energy particles, X- and gamma rays (refs, 5 27-31, 35, 40-42, 51, 63, 64, 68, 69, 71 and 73).

The biological effects of high-energy particles have also been under study since the 1950s by foreign investigators, at first for use in the treatment of neoplasms (refs. 75, 98, 100, 113, 120-122), later in connection with space research (refs. 57, 76, 78, 79, 81, 84 and 99).

The urgency of the problem led to holding special international symposia in the United States in 1962 and 1963 (refs. 78 and 79).

TABLE 3. THE RBE OF HIGH-ENERGY PARTICLES (LEL IN KeV/μ.

Recorded effect	Standard radiation		Protons (P), deutrons (D), alpha particles (α)		RBE	References
	Type	LEL	Type	LEL		
Destruction of yeast cells	X-rays 200 kV	2.9	D 190 MeV	0.9	1.0	(57)
Same, haploid strain	X-rays 180 kV	3.0	P 130-660 MeV	0.3-0.7	0.76	(27)
Same	γ rays Co^{60}	0.3	P 130-660 MeV	0.3-0.7	1.0	(27)
Same, diploid strain	γ rays Co^{60}	0.3	P 660 MeV	0.3	1.0	(27)
Destruction of Salmonella typhi	X-rays and γ rays Co^{60}	0.3-3.0	P 152 MeV	0.6	1.0	(81)
Chromosome aberrations in Vicia faba	X-rays 180 kV	30	P 170 MeV	0.6	0.7	(99)
Cell growth, in vitro culture	X-rays 200 kV	2.9	α 910 MeV	0.4	1.0	(124)
Recessive sex-linked mutations in Drosophila melanogaster	X-rays 180 kV	3.0	P 660 MeV	0.3	1.0	(53)
$LD_{50/30}$ in mice	X-ray 100 kV	3.6	P 730 MeV	0.3	1.1	(76)
Same	X-rays 250 kV	2.6	P 730 MeV	0.3	0.8	(76)
Same	X-rays 180 kV	3.0	P 660 MeV	0.3	0.7*	(1)
Same	X-rays 180 kV	3.0	P 660 MeV	0.3	0.67	(29)
Same	γ rays Co^{60}	0.3	P 660 MeV	0.3	0.9	(29)
Same	γ rays Co^{60}	0.3	P 660 MeV	0.3	0.8*	(62)

(See footnote at end of table.)

TABLE 3 (Continued)

Recorded effect	Standard radiation		Protons (P), deutrons (D), alpha particles (α)		RBE	References
	Type	LEL	Type	LEL		
$LD_{50/30}$ in mice	X-rays 125 kV	3.6	P 440 MeV	0.3	0.7	(84)
Same	X-rays 200 kV	2.9	P 340 MeV or D 190 MeV	0.4-0.9	~ 1.0	(120)
Same	γ rays Co^{60}	0.3	P 130 MeV	0.7	0.8	(62)
Same	γ rays Co^{60}	0.3	P 126 MeV	0.7	0.7	(18)
$LD_{50/12}$ in mice	X-rays 250 kV	2.6	P 730 MeV	0.3	0.9	(76)
Same	X-rays 100 kV	3.6	P 730 MeV	0.3	1.3	(76)
$LD_{50/8}$ in mice	X-rays 250 kV	2.6	P 157 MeV	0.7	0.77	(80)
$LD_{50/6}$ in mice	X-rays 250 kV	2.6	P 730 MeV	0.3	1.2-1.4	(76)
Same	X-rays 100 kV	3.6	P 730 MeV	0.3	1.3	(76)
Death of mice "under the beam"	X-rays 180 kV	3.0	P 660 MeV	0.3	~ 1.0*	(1)
$LD_{50/30}$ in rats	X-rays 180 kV	3.0	P 660 MeV	0.3	0.7*	(1)
Same	γ rays Co^{60}	0.3	P 510 MeV	0.3	0.75	(17)
Same	γ rays Co^{60}	0.3	P 240 MeV	0.4	0.73	(18)
Same	γ rays Co^{60}	0.3	P 126 MeV	0.7	0.70	(18)
Death of mice after fractional irradiation	X-rays 180 kV	3.0	P 510 kV	0.3	0.8	(17)

TABLE 3 (Continued)

Recorded effect	Standard radiation		Protons (P), deutrons (D), alpha particles (α)		RBE	References
	Type	LEL	Type	LEL		
Death of dogs after single exposure	γ rays Co^{60}	1.3	P 510 and 240 MeV	0.3-0.4	1.14	(17)
Same	X-rays 180 kV	3.0	P 126 MeV	0.7	1.0	(17)
Death of dogs after fractional irradiation	X-rays 180 kV	3.0	P 510 MeV	0.3	1.0	(17)
$LD_{50/30}$ in monkeys	Not shown	-	P 730 MeV	0.3	1.4	(112)
State of bone marrow and blood in rats	X-rays 180 kV	3.0	P 660 MeV	0.5	< 1.0	(48, 49)
Changes in blood in rabbits	X-rays 180 kV	3.0	P 480 MeV	0.3	~ 1.0	(54)
Degeneration of bone marrow cells in mice	γ rays Co^{60}	0.3	P 660 MeV	0.3	0.9	(64)
Chromosome aberrations in mouse bone marrow cells	γ rays Co^{60}	0.3	P 660 MeV	0.3	0.9*	(63, 64)
Chromosome aberrations in rat liver cells	γ rays Co^{60}	0.3	P 660 MeV	0.3	~ 0.9*	(45)
Chromosome aberrations in mouse corneal epithelium	X-rays 180 kV,	3.0	P 126 MeV	0.7	0.67	(42)
	γ rays Co^{60}	3.0	P 660 MeV	0.3	0.9*	
Decrease in weight of mouse testes	γ rays Co^{60}	3.0	P 660 MeV	0.3	0.8	(12)
Decrease in weight of mouse testes and genetic effectiveness (dominant lethals)	X-rays Co^{60}	3.0	P 660 MeV	0.3	0.6*	(47)
	γ rays Co^{60}	3.0	P 660 MeV	0.3	0.85*	
Decrease in weight of rat testes	X-rays 180 kV	3.0	P 660 MeV	0.3	~ 1.0*	(74)
Same	γ rays Co^{60}	0.3	P 660 MeV	0.3	~ 0.8	(12)
Dominant lethals in male rats	X-rays 180 kV	3.0	P 510 MeV	0.3	0.7	(46)

*RBE coefficient calculated with correction for more precise dosimetry (ref. 28).

Table 3 presents experimental data on the RBE of protons, deutrons and high-energy α particles obtained through 1964 on a variety of biological objects. Evaluation of the published data is quite difficult owing to the relative complexity and variety of the methods used to measure high-energy particles. In the works of Soviet authors, the specific absorbed dose of 660 MeV of protons was 5.3 (ref. 1), 4.35 (ref. 5), and $4.0 \cdot 10^{-8}$ (ref. 28) rad·cm^2/proton. The foreign investigators, unfortunately, fail to report this value. In table 3, a correction was introduced into the data on the RBE of 660 MeV of protons in accordance with the value of the specific absorbed dose of $4.0 \cdot 10^{-8}$ rad·cm^2/proton obtained in special experiments performed by Konoplyannikov (ref. 28).

It was possible, therefore, to compare the experimental data of different authors. Otherwise, the differences in dosimetry would reach and sometimes even overlap the value of the RBE for different indices.

It is evident from table 3 that the RBE of high-energy particles is in most cases < 1.0, resembling the relationship between high-energy gamma or X-rays and standard X-rays (table 1). The RBE of protons with an energy of 130-730 MeV in relation to the effects of standard X- or gamma rays taken as 1.0 generally fluctuates between 0.6-0.8 or 0.9-1.0, respectively. On the whole, this satisfactorily correlates with the LEL values. /148

The fact that the biological effectiveness of high-energy protons is lower than that of gamma rays may be due to the difference in dose rate and to ultrafractionation of the radiation, as noted by other authors as well (refs. 1 and 54). This difference in effectiveness between protons and gamma rays did not occur in experiments on isolated cells. The investigation on two strains of yeast cells showed that 660 MeV of protons and Co^{60} gamma rays was equally effective in a broad range of doses. This was observed both during analysis of the inactivation of cells from total destruction and from the types of injury to the yeast cells formed by different kinds of microcolonies (ref. 32, figs. 1 and 2).

The previously noted growth in the value of the RBE of harder radiation with increase in size of the irradiated animal also occurs with proton radiation of monkeys and dogs. The increase in the RBE coefficient for high-energy particles is caused, however, both by the greater penetrating power and by the nature of the distribution of LEL, specifically by increase in the LEL of secondary particles deep in the animal's body, as other investigators have pointed out (ref. 51).

Thus, analysis of the data presented in table 3 shows that the /150 relationship between the RBE of high-energy particles and the LEL duplicates the familiar pattern of radiations with low specific ionization.

The only slightly dissonant note here is introduced by experiments performed on rats. Judging by the data of different authors, the RBE of high-energy protons according to $LD_{50/30}$ of rats is 0.7-0.75, relative to X- or gamma rays

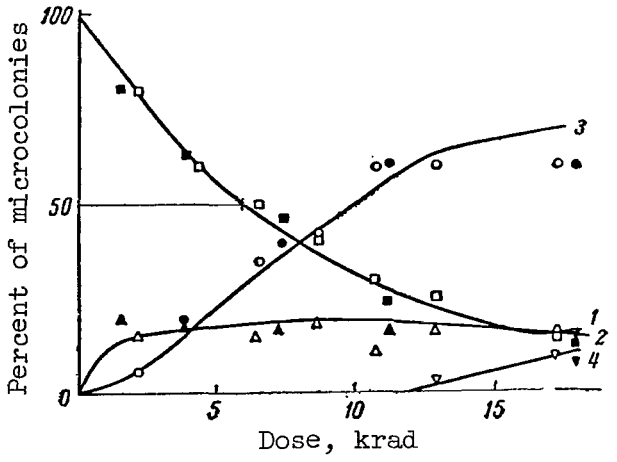

Figure 1. Formation of different kinds of microcolonies of haploid yeast cells after proton and gamma irradiation. 1, 2, 3, 4, normal microcolonies; inactivation after 2-7 divisions, inactivation after 1-2 divisions, destruction without division (ref. 32), respectively; black, Co^{60} rays; light, protons of 660 MeV.

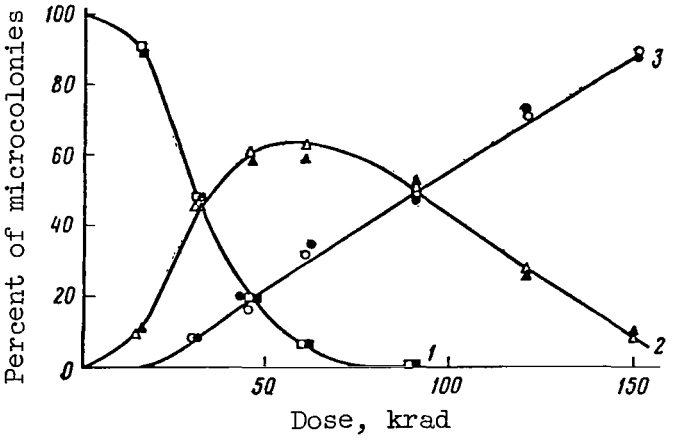

Figure 2. Formation of different kinds of microcolonies of diploid yeast cells after proton and gamma irradiation. Symbols same as in figure 1.

(ref. 17). However, from table 2 we might have expected that the RBE of protons would be somewhat higher than unity, relative to X-rays, and would approach unity in comparison with gamma rays.

TABLE 4. SURVIVAL RATE OF RATS IRRADIATED WITH PROTONS, ACCORDING TO THE DATA OF DIFFERENT AUTHORS.

Proton energy, MeV	Indices	Dose, rad (published source) (1)	(51.17)*	(6.51)
660	$LD_{50/30}$	760	-	-
	$LD_{100/30}$	1050	-	-
510	$LD_{50/30}$	-	580	-
	$LD_{100/30}$	-	830	430-550

*For Wistar strain rats, which according to the literature (ref. 50), are more radiosensitive than white stock-bred rats (by ~ 10-15%).

Referring to the data presented in table 4, we see that the RBE is difficult to evaluate because of the great differences in the experimental results, the quantity of which is still insufficient for any definite conclusions.

3. Characteristics of the Biological Effect of Radiations with Low Specific Ionizations

Regarding the survival rate of irradiated mice, there is some decrease in the mean lifespan after gamma or proton irradiation as compared with that observed after exposure to standard X-rays. For example, the mean lifespan of mice irradiated with a minimum absolutely lethal dose (~ 700 rem) is /151 10-12 days for X-rays and 6-9 days for high-energy protons or gamma rays. The mean lifespan integrally characterizes the death of animals at various intervals after irradiation, which reflects, in turn, the systemic nature of radiation injury (refs. 11, 38, 39 and 52). The hematopoietic organs (~ 800 rem) and intestines (> 1000 rem) are the critical organs in a range of 600-1300 rem. By analyzing from this standpoint the correlations of the minimum absolutely lethal doses during 30 and 4 day observations, we see that as the LEL increases, the interval between the doses that cause death at these times also increases (table 5).

A similar conclusion on the correlation of "intestinal" and "bone marrow" death in mice was reached independently for protons of 730 MeV (ref. 76).

For a quantitative description of the degree of manifestation of "bone marrow" and "intestinal" death after exposure to different radiations of low specific ionization, we determined the constant K, which is the ratio of $LD_{100/4}$ to $LD_{100/30}$ in mice (table 5). The value of K is approximately the same for protons and gamma rays. This seems to be due to the similar values of the LEL and approximately the same distribution of absorbed energy in different organs. The difference in degree of radiation vulnerability of the intestine and

TABLE 5. DETERMINATION OF CONSTANT K FOR DIFFERENT RADIATIONS OF LOW SPECIFIC IONIZATION.

Ionizing radiation	$LD_{100/30}$, rad	$LD_{100/4}$, rad	$K = \frac{LD_{100/4}}{LD_{100/30}}$
Protons of 660 MeV	950-1000	1350-1450	1.4
Co^{60} γ rays	850-900	1200-1300	1.4
X-rays	700-750	1100-1300	1.7

hematopoietic organs (bone marrow in particular) after exposure to gamma rays and protons of 660 MeV, on one hand, and to X-rays, on the other, is attributable to the different correlation of the absorbed doses of energy in these systems, similar to that described above for the radiation death of mice and rats (table 4).

In the case of X-rays, the relative significance of injury to hematopoiesis increases because of the greater injury to bone marrow, due to the nature of energy absorption in bone as compared with soft tissues (ref. 114). Consequently, here too, change in the value of K reflects not a difference in radio- /152 sensitivity of the intestines and hematopoietic organs to the comparable radiations of low specific ionization, but the nature of the distribution of the absorbed doses in these organs.

The study of RBE is not limited to a quantitative description of the radiations in question. It is equally important to determine the qualitative characteristics of their biological effects. The matter is particularly urgent in evaluating the RBE of radians of low specific ionization. In the earliest investigations on mice receiving whole-body radiation with deutrons (190 MeV), the authors concluded, after analyzing the clinical symptoms of the injury and results of blood tests, that the effects were identical to those produced by X- or gamma rays (refs. 120 and 122). Ten years later the same investigators performed detailed comparative radiobiological experiments with deutrons (190 MeV), protons (315 MeV), and X-rays (180 kV) and confirmed their earlier conclusion. They also demonstrated a distinct relationship between the RBE of these radiations and their LEL (ref. 120).

Soviet investigations (including our data and derived from a great variety of indices) failed in most cases to reveal any special characteristics of the radiobiological effects of high-energy protons, namely, the results of hematological studies (refs. 48, 49 and 54), cytological analysis of radiation injury to bone marrow, including the study of early degenerative changes (interphase death of cells), level and dynamics of change in the percentage of cells with chromosome aberrations and mitotic activity (refs. 45, 63 and 64), analysis of changes in blood protein fractions (ref. 13), dynamics of

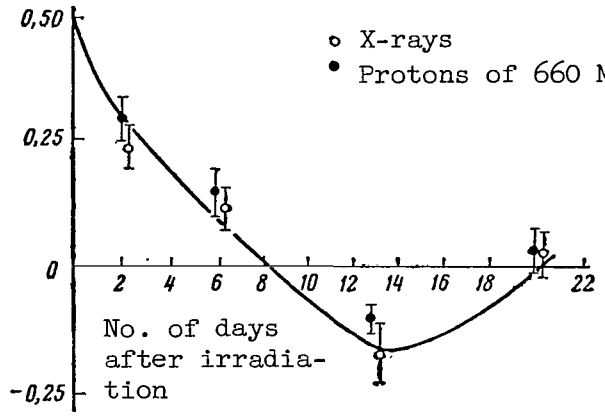

Figure 3. Change in extent of "residual injury" in relation to time elapsing between first and second exposures.

weight of testes and restoration of germ cells (refs. 12 and 47), degree of loss of reproductive function (ref. 71) and formation of dominant lethals (refs. 46 and 47).

Our recent experiments on the dynamics of restoration of the radioresistance of mice by Blair's method (ref. 9) (which permits integral evaluation of the course of injury and repair under the influence of certain radiation agents) also show that the effects produced by high-energy protons and X-rays are identical. As an indicator of the degree of radiation injury we used the mortality rate of mice during the 30 days following exposure to protons of 660 MeV or X-rays of 180 kV. After determination of the $LD_{50/30}$, which came to

890 ± 33 and 590 ± 22 rad, respectively, two groups of mice were irradiated with a dose of 0.5 $LD_{50/30}$, i.e., with doses of 445 and 295 rad, respectively,

of proton and X-rays. The animals were X-rayed again 2, 6, 13 and 20 days after the first exposure to determine the $LD_{50/30}$. The extent of /153

residual injury between the first and second exposures was expressed as the difference between the values of the $LD_{50/30}$ after single and repeated irradia-

tion. It is evident from figure 3 that residual injury decreased rapidly during the first week after irradiation, reaching zero in 8-10 days. This was followed by a period of increased radioresistance with the maximum 12-14 days after irradiation; by the twentieth day radioresistance was indistinguishable from what it was at the beginning.

The period of semirestoration was ~ 2.5 days for protons of 660 MeV and ~ 2.0 days for X-rays. This difference, like that in the dynamics of restoration after exposure to both types of radiation, was statistically insignificant throughout the period of injury.

Bak and Aleksander (ref. 10) conjectured that postradiation restoration in this dose range is determined chiefly by bone marrow capacity for regeneration, the rate of which varies with the extent of the initial injury as determined by the amount of absorbed radiant energy. Recent experimental studies have confirmed this hypothesis (refs. 66 and 109). In this case the general character of the repair rate was established for protons of 660 MeV and X-rays.

Convincing data on the complete identity of acute radiation injury caused by protons of 510 MeV and X-rays were obtained in experiments on dogs and cats (ref. 8), designed to study the survival rate, hematopoeisis and the cardiovascular system, gas exchange, immunobiological reactivity, protein composition, blood serum cholinesterase activity and pathomorphological /154 changes. The data completely confirmed the earlier findings in mice and rats (ref. 43). Fractional irradiation of dogs with protons (510 MeV) produced a pronounced hemorrhagic syndrome, which the authors tend to regard as a characteristic of proton irradiation (refs. 7 and 36). However, in experiments with repeated X-irradiation of dogs comparable in dose and time distribution to proton irradiation, the hemorrhagic syndrome was just as pronounced.

The most logical explanation of the phenomenon is found in the delayed death of the animals exposed to fractional irradiation (between 40 and 60 days), which resulted in the hemorrhagic syndrome being manifested in all its diversity, whereas after a single exposure the animals died between 8 and 14 days with symptoms of severe leukopenia. We cannot rule out the differences in evaluation of the cumulative effects of irradiation on the hematopoietic organs and systems responsible for the development of the hemorrhagic syndrome. The high reparative capacity of the former is well known, whereas the latter have not yet been studied in this respect.

Some light was thrown on the specific effect of protons by a detailed description of the chromosome injuries observed in resting liver cells (ref. 45) after hepatectomy and in dividing corneal cells (ref. 42). Under the influence of protons of 660 MeV, the number of liver cells with bridges and bridges with fragments is greater than after the same dose of gamma irradiation (30.5 ± 3.1 and 15.7 ± 2.3 percent, respectively, with $t = 3.8$ and $P < 0.1$), although the number of fragments per injured cell does not change (1.2 ± 0.08 and 1.3 ± 0.05). On the other hand, on the basis of an analysis of the curve reflecting the production of cells with pathological mitoses in the cornea, it has been conjectured that irradiation with 126 MeV of protons gives rise to a relatively large (as compared with the effect of X-rays) number of cells with chromosome aberrations possessing high reproductive capacity, thus enabling them to remain longer in irradiated tissue. This type of injury to the nuclear apparatus may explain, in part, the increased blastomatogenic effect of high-energy protons (ref. 59), the origin of which is still difficult to account for without considering the possible role of the heavy component, which arises during the nuclear interactions of the protons (refs. 5 and 28). However, the greater carcinogenic effect of high-energy protons as compared with that of X-rays requires further study, chiefly in quantitatively adequate comparative experiments (ref. 41).

Thus, comparative analysis of most of the direct biological effects of high-energy protons shows them to be identical to those of X- or gamma rays. The nature of the manifestation of radiation injury of animals, espe- /155
cially large ones, is largely determined by the distribution of the absorbed energy in the various organs and systems. Therefore, application of the general principles of prevention and therapy worked out for lesions caused by radiations with low specific electromagnetic ionization to the prevention and treatment of lesion caused by high-energy corpuscular radiation seems to us to be wholly warranted.

4. Comparative Effectiveness of Chemical Radioprotective Agents against X-, Gamma and Proton Irradiation

The possibility of significantly increasing radioresistance to irradiation with high-energy protons by means of protective agents was demonstrated for the first time in 1961 (refs. 68 and 70) in the case of the aminothiols and indolyl alkylamines, whose effect is believed to be achieved by different mechanisms. It was then shown that after irradiation of mice with absolutely lethal doses of high-energy protons, up to 50 percent of the animals survived if the extremities were ligated during exposure, as was established in earlier experiments with X-rays (refs. 24, 25 and 37). This phenomenon is based on the preservation of some bone marrow cells in the ischemic part of the femur below the ligation. Consequently, after irradiation with high-energy protons there is complete manifestation of the oxygen effect, as in all cases of exposure to radiations with low specific ionization.

Several compounds were compared and found to be equally effective against proton, X- and gamma irradiation (ref. 65). Later both Soviet (refs. 4, 30 and 62) and foreign (ref. 107) investigators confirmed the protective activity of some chemical agents against high-energy protons.

Table 6 lists the compounds tested, together with some new data of our own. An attempt was made to characterize quantitatively the degree of effectiveness against some radiations with low specific ionization and the manifestation of chemical protection in relation to the LEL of the radiation and its penetrating power.

It is evident from table 6 that the aminothiol and indolyl alkylamine derivatives are the most effective protectors against the three kinds of ionizing radiation studied. The degree of protection against radiation in minimum absolutely lethal doses (700, 850 and 1000 rad for X-rays, gamma rays and protons, respectively) differs little regardless of the type of radiation. Very cautiously we may say that the indolyl alkylamines seem to be somewhat less effective against proton and gamma irradiation, possibly because they are less effective against radiation lesions of the intestines (refs. 16, 67 and 104), which as mentioned before, occur quite often in these cases. However, definitive conclusions will require special comparative experiments with /157
determination of the FDD value (factor of decrease in dose) for specific protectors in a wide range of doses embracing the different forms of systemic lesions. Table 6 shows that fluctuations even in some of our own experiments

TABLE 6. COMPARATIVE EFFECTIVENESS OF PROTECTIVE AGENTS AGAINST RADIATIONS WITH LOW SPECIFIC IONIZATION.* (Published Sources in Parentheses.)

Protective agents, conditions of use	X-rays of 180 kV, 670-720 rad		Co^{60} γ rays, 810-860 rad		Protons of 660 MeV, 950-1,100 rad	
	No. of mice	% surviving	No. of mice	% surviving	No. of mice	% surviving
1	2	3	4	5	6	7
Control	120	5	115	2	140	2
	-	-	120	0 (62)	160	1;7 (62)
Cysteamine hydrochloride, 150 mg/kg	40	60	50	70	70	60
Cysteamine dihydrochloride, 150 mg/kg	30	46	30	30 (15)	46	95
			40	55 (62)	30	57 (4)
					80	51 (62)
AET dihydrobromide, 150 mg/kg	89	62 (62)	30	40 (65)	68	85
			35	91 (72)	30	84 (4)
			40	75 (62)	60	81 (62)
l-cysteine hydrochloride, 1,000 mg/kg	20	70	-	67 (39)	15	73
2-aminothiazoline hydrobromide, 150 mg/kg	-	35 (61)	-	-	15	60
3-aminopropylthiosulfuric acid, 250 mg/kg	50	50 (3)	-	-	15	27
5-hydroxytryptamine creatinine sulfate, 75 mg/kg	80	50 (33)	40	60 (62)	54	43
					30	43 (4)
					30	50 (62)
5-methoxytryptamine hydrochloride, 50 mg/kg	20	65 (65)	20	45 (65)	69	48
	40	90 (67)	20	75 (72)	30	60 (4)
	75	68 (33)	40	70 (62)	30	70 (62)
Tryptamine hydrochloride, 100 mg/kg	30	4	20	20 (62)	20	15 (4)
	30	36 (33)				
Epinephrine, 5 mg/kg	-	-	-	-	15	
Histamine dihydrochloride, 300 mg/kg	30	30	-	-	15	27
Hydroxylamine hydrochloride, 60-75 mg/kg	40	16-60 (92)	25	4	39	20

(See footnote at end of table.)

TABLE 6 (Continued)

Protective agents, conditions of use	X-rays of 180 kV, 670-720 rad		Co^{60} γ rays, 810-860 rad		Protons of 660 MeV, 950-1,100	
	No. of mice	% surviving	No. of mice	% surviving	No. of mice	% surviving
1	2	3	4	5	6	7
Potassium cyanide, 4 mg/kg	-	30 (123)	-	-	15	33
EDTA, 100 mg/kg	-	-	-	-	15	20
ATP, 250 mg/kg	-	-	-	-	15	40
Testosterone propionate, 100 mg/kg (for 14 days)	-	-	-	17-29 (19)	20	35

*Solutions of the compounds of injected intramuscularly 5-20 min before irradiation (except testosterone propionate).

performed at different times are quite large. It is thus impossible to evaluate quantitatively the relationship between the degree of manifestation of the protective effect and the LEL in the range under study (0.3-3.0 KeV/μ).

Of independent theoretical and practical interest are the attempts to find ways of intensifying the radioprotective effect by combined use of protectors which have a presumably different mechanism of action (refs. 26, 34 and 65), or which mainly protect certain radiosensitive organs (refs. 72 and 105). It is evident from table 7 that this possibility also holds for proton irradiation. However, here we must also abstain from trying to establish any quantitative relationships until appropriate comparative studies are made. /158

The first reports have recently appeared on the use of protective agents against proton irradiation of rats and dogs (ref. 6). In experiments involving a small number of animals, the authors found that cystamine and 5-methoxytryptamine had no protective action, although AET and 5-hydroxytryptamine enabled half of the experimental animals to survive. In the same work, most of the protective agents (AET, cystamine, 5-methoxytryptamine, serotonin, PAPP) were ineffectual against gamma irradiation of mice with a dose of 600 r. The inconsistency of these findings with the numerous published data (refs. 56 and 93) may be due to the experimental conditions (doses and methods of application). It is difficult to evaluate the experiments on rats due to the previously noted lack of definite information about the lethal doses of protons for these animals (table 4) or the experiments on dogs because of fractionation of the irradiation and subsequent therapy used. /159

TABLE 7. EFFECTIVENESS OF COMBINED USE OF PROTECTIVE AGENTS. (Published Sources in Parentheses.)

Dose, rem	Compounds and doses	X-rays of 180 kV		Co^{60} γ rays		Protons of 660 MeV	
		No. of mice	% surviving	No. of mice	% surviving	No. of mice	% surviving
700-750	Cysteamine, 150 mg/kg + 5-methoxytryptamine, 75 mg/kg.	50	92 (34)	18 19	95 (65) 95 (72)	33	67
1,000	Same.	40	27 (34)	-	-	40	50
	Cysteamine, 150 mg/kg . .	20	0 (34)	-	-	20	10
	5-methoxytryptamine, 75 mg/kg.	20	0 (34)	-	-	20	0
950	Cysteamine, 150 mg/kg + serotonin, 75 mg/kg .					30	53
	Cysteamine, 150 mg/kg . .			-	-	15	33
	Serotonin, 75 mg/kg . . .			-	-	15	13
750	Hydroxylamine, 60 mg/kg + AET, 150 mg/kg. . . .	20	90 (92)	27	81	31	87
950	Cysteamine, 150 mg/kg + potassium cyanide, 2 mg/kg	-	-	-	-	15	40
	Cysteamine, 150 mg/kg . .	-	-	-	-	15	33

The relationship between degree of modification of the radiation effect and the LEL can be readily analyzed in standard experiments, particularly on microorganisms. Tables 8 and 9 summarize the results of experiments on diploid and haploid yeast cells irradiated with Co^{60} gamma rays and protons (660 MeV) accompanied by the use of anoxia and various chemical compounds.

It is clear from the foregoing that the degree of manifestation of protection afforded cells of both strains was the same for proton and gamma irradiation. The maximum effect was noted under the influence of anoxia, glycerin and sulfur-containing compounds. The biological amines had virtually no effect on the survival rate of the irradiated cells, a fact which is consistent with current views on the pharmacological nature of their mechanism of action (refs. 15, 23 and 58). Thus, for most biological objects, including

TABLE 8. EFFECTIVENESS OF PROTECTION AGAINST IRRADIATION OF DIPLOID YEAST CELLS.

Protective agent	Concentration	FDD (factor of decrease in dose)	
		Co^{60} γ rays	Protons of 660 MeV
Anoxia	< 0.2 mm Hg	2.8	2.8
Glycerin	6.5 M	2.2	2.2
Cysteamine hydrochloride	10 M	2.1	2.1
Cysteamine hydrochloride	10^{-2} M	1.7	1.7
Cysteamine dihydrochloride	10^{-2} M	1.6	1.6
Hydrosulfite	10^{-2} M	1.5	1.5
l-cysteine hydrochloride	10^{-2} M	1.4	1.4
S, β-aminoethylisothiuronium bromide hydrobromide	10^{-2} M	1.3	1.4
Sodium salt of fumagillin	10 mg/ml	1.4	1.4
Hystamine chloride	$5 \cdot 10^{-1}$ M	1.3	1.3
Ethyl alcohol	1.2 M	1.3	1.3
Ascorbic acid		1.3	1.3
Hypertonic solution of sodium chloride	1.7 M	1.3	1.3
Glucose	$3.6 \cdot 10^{-1}$ M	1.2	1.2
Vinylpyramidon	$2.2 \cdot 10^{-1}$ M	1.2	1.2
Streptomycin sulfate	100 γ/ml	1.2	1.2
5-hydroxytryptamine creatinine sulfate	10^{-2} M	1.1	1.1

TABLE 8 (Continued)

Protective agent	Concentration	FDD (factor of decrease in dose)	
		Co^{60} γ rays	Protons of 660 MeV
5-methoxytryptamine hydrochloride	10^{-2} M	1.0	1.0
Sodium cyanide	$5 \cdot 10^{-3}$ M	1.0	1.0
Propylgallate	10^{-2} M	1.0	1.0
Sodium oleate	$4 \cdot 10^{-2}$ M	1.0	1.0
Gelatin	0.5 %	1.0	1.0
Serum albumin	0.5 %	1.0	1.0

TABLE 9. EFFECTIVENESS OF PROTECTION AGAINST IRRADIATION OF HAPLOID YEAST CELLS.

Protective agent	Concentration	FDD (factor of decrease in dose)	
		Co^{60} γ rays	Protons of 660 MeV
Anoxia	< 0.2 mm Hg	2.6	2.6
Glycerin	6.5 M	2.3	2.3
Cysteamine hydrochloride	10^{-2} M	2.0	2.0
Cysteamine hydrochloride	10^{-3} M	1.4	1.4
S, β-aminoethylisothiuronium bromide hydrobromide	$5 \cdot 10^{-3}$ M	1.4	1.5
5-hydroxytryptamine creatinine sulfate	10^{-2} M	1.0	1.1
Sodium cyanide	10^{-3} M	1.0	1.0

small laboratory animals (mice), the RBE of radiations with low specific ionization characterized by LEL of the order of 0.3 KeV/μ (high-energy X-rays, gamma rays and high-energy protons) relative to standard X-rays, ranges from 0.5-0.9, depending on the indices studied. With increase in the linear size /160 of the biological objects, the value of the RBE of the aforementioned radiations approaches unity or even slightly exceeds it. This is primarily due to the energy distribution of the radiation in the various organs and systems.

A comparative analysis of a great many indices of the biological effects of high-energy protons, gamma and X-rays shows that at least for the direct effects the radiation injuries are completely identical, especially those caused by proton and gamma irradiation, which are characterized by similar LEL values.

The results of experiments involving the use of modifying agents at the cellular and organismic levels confirm the fact that the LEL value plays an important part in the radiobiological effects of ionizing radiations, regardless of their physical nature (ref. 110).

In view of the increasing need for detailed information on different radiation conditions and on the influence of irradiation combined with dynamic spaceflight factors, we recommend, in general, the use of gamma rays or, in some cases, X-rays instead of the cumbersome and costly experiments on high-energy particle accelerators. This approach also yields the most precise quantitative data because of its simplicity and convenience and because it permits greater methodological rigor.

The relationship between the RBE and biological effects of high-energy particles is still unclear in some respects. Further research is needed in the following direction:

(1) quantitative evaluation of various modifying agents;

(2) specific information on injury and repair at the cellular level, including a detailed analysis of injury to hereditary structures;

(3) evaluation of the significance of secondary radiations arising during proton irradiation of large animals;

(4) obtaining more precise data on the long-term effects of irradiation with high-energy particles.

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NEW ASPECTS OF PERSONAL HYGIENE

V. V. Levashov

The chapter on personal hygiene in textbooks and manuals has remained /165
virtually unchanged for the past hundred years. This chapter has always been more modest than the others in both size and content.

Conditions have now arisen that justify broadening and deepening it. The section on hygiene should be extended just as some sections of physics and chemistry, once thought to be complete, have been extended and revised.

The new conditions are the result of manned spaceflights. These flights are associated with unusual external circumstances which affect the physiologico-hygienic indices of human activity. The metabolic processes, the status of protective forces within the human body and the biological properties of microorganisms may change significantly during prolonged flights. Thus, personal hygiene becomes unusually important with a variety of purposes--physiological, hygienic, epidemiological and psychological.

It is no exaggeration to say that the specific features and breadth of personal hygiene in space are at a wholly different level from that on Earth.

According to F. G. Krotkov, personal hygiene embraces the hygienic maintenance of the body, clothing and footwear, bedding and other objects used in daily living and while at work. The purpose of personal hygiene is to preserve and strengthen health.

The concept of hygienic maintenance of the body in this classical definition is incomplete and for the purposes of space medicine it must be filled out /166
in detail.

The scientific determination of personal hygiene measures required for cosmonauts must start with complete knowledge of the functioning of the human skin and its influence on the physiological processes.

In the extensive literature on human physiology and the mass of clinical materials of dermatology, the section dealing with the quantitative indices of the functioning of the human skin is very small. Thus, much remains to be done in this field.

It is quite apparent that there is a need for scientific study of the optimal and permissible values of the biological, physiological and chemical indices

of the skin in its interrelationship with the organism as a whole. These indices will undoubtedly have to be differentiated with respect to the various external conditions of spaceflight (extent of change in the gravitational field, composition of gases, nature of human activity, etc.).

Recent published reports detailing the results of research on individual indices and functional levels of the human skin are of undoubted value in developing the principles of personal hygiene for cosmonauts.

It is evident from the Soviet and foreign literature that halting or shortening normal toilet routines impairs the sense of well-being by the end of the second week. The situation gradually becomes worse, sometimes resulting in skin changes or even diseases. People are particularly affected by the inability to wash their head, shave, or brush their teeth.

The development of space medicine and biology has led to a variety of experimental investigations conducted by Soviet and foreign scientists in special chambers (pressure, soundproof, thermal). These chambers are comparatively small and lack natural light. Limited mobility and absence of ultraviolet radiation undoubtedly had an adverse effect on some biological indices, as in the case of spaceflight. Among these investigations are studies on several physiological, chemical and bacteriological indices of the skin of human beings confined to a small space for a long period of time.

For example, V. F. Talapin found that the elasticity (deformity) of some portions of human skin is greatly altered, by about twice as much as the /167 original value. Significant changes in this physiological index may have negative psychological effects as well as restricting the scope of functional movements.

It is no accident that skin elasticity, according to A. P. Parfenov, is considerably changed in middle-aged and elderly people and in those doing little physical work. The mechanism of these reversible changes has not been elucidated, but it is reasonable to assume that one of the factors is the abnormal functioning of the sebaceous and sweat glands. The functions of the human sweat glands have been extensively studied (notably in the detailed monograph of Kuno), but very little attention has been paid to the sebaceous glands. Worth noting here is the investigation of A. M. Finogenov, who used original methods (in 1963) in long-term experiments on human beings. According to this author, there is a steady accumulation of sebum on exposed skin after five days--440 to 470 mg/cm^2, far in excess of the original values.

Sebum formation on exposed skin then stabilizes. At the same time, Finogenov found little change in the accumulation of sebum on the covered parts of the body during this time (about seven days), because it is continuously sorbed by the fabric of the clothing. Later, however, the sorption properties of the clothing decrease.

Investigations on changeability of skin elasticity and sebum formation are the basis for a fuller understanding of the biological processes that take place

in human skin under special conditions. They also constitute a starting point for determining the qualitative and quantitative characteristics of hygienic procedures.

One of man's defense mechanisms is the bactericidal function of the skin. Although it has been known to medicine for many years, the biological aspects have by no means been thoroughly studied. The antimicrobial function of the skin is particularly important during spaceflight, because it largely determines the biological balance between the cosmonauts and their constant fellow-travelers, the microbes. It is also responsible for epidemiological protection, and it may well be a factor in the possible biological danger involved in landing on other planets.

According to M. I. Kozar', prolonged confinement of human beings in a small space with limitations on or prohibition of hygienic care impairs the dis- /168
infectant function of the skin.

Thus, seeding the surroundings (clothing, air, room) with microbes may, in the light of N. N. Klemparskaya's studies, create a serious danger of endogenous infections. To protect the cosmonauts from the accumulation of microorganisms in and on them, we must work out a set of prophylactic measures, with a prominent place accorded to disinfection of the atmosphere and treatment of the skin and mucosa. We cannot agree with V. I. Vashkov, who believes that the first step should be to search for ways and means of reducing the number of microorganisms.

Returning to the defense mechanisms of the human body, attention should be paid to the existence of a possible relationship between the bactericidal functions of the skin and such indices as the nonspecific reactivity of the skin and variability of the chemical composition of sebum.

According to V. V. Ogleznev, prolonged confinement of human beings in a small space prevents the skin from resisting external factors (change in nonspecific reactivity of the skin). A. M. Finogenov maintains that the action of oxygen, moisture, skin lipase and enzymes released by microorganisms increases the amount of free higher and lower fatty acids, ketones and aldehydes. We believe that these facts have to be considered in analyzing the intimate relations between micro- and macroorganisms. Specifically, further analysis of the connection between increase in acidity of the contaminated skin surface (A. M. Finogenov) and simultaneous change in growth of microorganisms after a lowering of the protective function of the skin (M. I. Kozar') is particularly important.

In accordance with the well-known studies of P. A. Alisov on the positive role of the symbiotic microflora, we surmise that personal hygiene measures for the cosmonauts will not adversely affect their autoflora.

In conclusion, we wish to point out that the development of personal hygiene measures (from cleaning the skin to preserving its biological constants) is a field in itself.[1] The complexity and uniqueness of the technical methods involved stem from the fact that they must be effective under the conditions of an altered gravitational field and adequate in the presence of minimum water consumption.

[1]Suffice it to say that the leaking of liquids in weightlessness is unusual to our terrestrial ideas.

SOME ASPECTS OF HUMAN ECOLOGY IN CLOSED SYSTEMS WITH RECIRCULATION OF SUBSTANCES

Ye. Ya. Shepelev

Prolonged manned spaceflights and the organization of habitable /169
planetary and orbital stations require, in principle, new methods of creating living conditions in the spacecraft and selecting ways and means of ensuring the vital activity of the crew.

The most promising approach to the problem involves reducing the amount of essential substances (oxygen, water, and food) carried on board to a minimum and using them over and over again. This can be done only by cyclic renewal of these substances in a closed ecological system, in which the products of man's vital activity are transformed by other living organisms into a primary form acceptable to man.

The initial link in these transformations is the decomposition of organic compounds into minerals consumed by plants. In nature this process is carried out by soil and water microorganisms. In the course of photosynthesis, green plants take up carbon dioxide, release oxygen, and synthesize organic substances, the basis of the vegetable part of man's diet. Some of the plant biomass is secondarily transformed by heterotrophic organisms into animal proteins and fats. This permits the creation of the physiologically necessary variety of food sources to maintain an adequate diet for man.

The organic substances assimilated in the human organism release their stored energy and in the form of products of man's activity enter the next cycle, which may continue until the quantity of some of the circulating substances appreciably diminishes, due to the noncyclic processes inevitably present in the model and which remove some of the substances from the cycle. Scientists /170
have now described both the general principles and variations possible in the design of life-support systems on the basis of a biological cycle of matter, in which each stage is realized by different groups of microorganisms, algae, higher plants and lower and higher animals (refs. 10, 12, 14, 22, and 23).

This article examines some of the problems that may confront biologists and hygienists in planning the maintenance of human activity on the basis of closed systems with biological recirculation of substances.

The natural striving to ensure the habitability of a spacecraft by the regeneration of vital substances instead of stocking them for the entire flight makes the life-support measures a closed thermodynamic system in which man

becomes a constituent, one of its functional links. The principle underlying this method is that human activity is maintained not by creating independent facilities, but by including man himself as a constituent element in a system of material-energy relations capable of existing for a long time as an autonomous unit. Man here is an object to be safeguarded only insofar as he can ensure the normal functioning of the other links in the system. This demonstrates, among other things, the tentativeness of the term "life-support system" as applied to the closed ecological systems that include man.

In view of these characteristics of man's position in a closed system, it is particularly important to pose the problem of human ecology as a general biological discipline, the objective of which is the entire set of man's material-energy relations with the environment and its constituent parts, where the nature of the environment is not only a condition but a function of the physiological processes in the human organism. The tasks of human ecology are now handled largely by applied physiology and hygiene. They include general, communal and food hygiene, physiology and hygiene of work and rest, industrial toxicology and some branches of physiology and pathology and other sciences concerned with the interrelations of man and his working and living conditions.

The physiological and hygienic aspects of the man-environment problem have thus far been studied largely by investigation of the one-sided demands made by man on the totality of factors of an artificial environment, as they affect his living and working conditions. The reverse influence of man on the environment has been examined mostly in connection with pollution of the atmosphere by respiratory products and other gaseous admixtures released as a result of /171 human activity. This aspect of the problem has considerable practical importance, because human beings must remain confined in small isolated quarters for a long period of time (submarines, various kinds of airtight installations on the ground).

With respect to habitable space vehicles capable of existing independently for a long time, the tasks of studying and providing hygienic normalization of environmental factors have been growing in scope and variety. This has made it necessary to review or determine more precisely most of the known hygienic norms and environmental requirements of man, with due regard for the prolonged action of the environmental factors, on one hand, and for the need to maintain man's efficiency, on the other.

The peculiar problems involved in normalizing an artificial environment in closed systems emerged from the necessity to know not only the optimum values but also the permissible limits of transient and prolonged deviations from comfort indices. This is due to the extended independent existence of the crew in low-capacity systems and to the specific nature of the regeneration systems operating in a closed cycle with much larger fluctuations than those characteristic of open systems, e.g., in systems with biological recirculation of substances. Here, in addition to the usual problems involved in the hygiene of airtight places, considerable importance is attached to investigating the dynamic characteristics of the set of environmental factors as a whole, because in closed systems with recirculation of substances all aspects of the process are interrelated, where a change in one of the environmental components changes the state of the system as a whole.

The functional mobility of systems with the recirculation of substances is based primarily on the ordinary functional changes in the living components of the system, which lie within the physiological norms of their reaction to changes in the surrounding conditions or which display their own physiological rhythms (diurnal, seasonal). An illustration of this is the ordinary physiological dynamics of human gas exchange in relation to the current value of physical activity and its daily rhythm, which directly affects, for example, the function of photosynthesizing organisms, the consumers of carbon dioxide in a biological air regeneration system. To these comparatively brief fluctuations we must add the more persistent changes in the normal functional level of individual components of the system in response to longer or frequently recurring unidirectional changes in individual environmental factors. We are now dealing mostly with less reversible changes in the initial functional characteristics of the living components of the system, with an adaptive shift of the /172
physiological norm.

Thus, the activity of a system with the recirculation of substances is reflected in continuous changes in the current value of all functional parameters of the environment, which constitute a complex and variable function of the fluctuations in each of the links, both inherent and dependent on external conditions. As a result, the complex of environmental factors in systems with biological recirculation of substances is theoretically the least stable of all life-support systems.

The scientific principles involved in normalizing the environment in accordance with the specific conditions of spacecraft were worked out in detail by A. M. Genin (ref. 3). All we need add here is that it is necessary to define more precisely the criteria for combining thermal environmental factors with various levels of atmospheric pressure, because it has been shown that low atmospheric pressure intensifies heat emission as a result of evaporation from the skin, thereby changing the thermal balance and shifting the heat comfort zone (ref. 4).

One of the important aspects of human ecology in closed systems calls for the investigation of new environmental factors related to specific links in the regeneration and circulation of substances. It is at this point that human ecology comes closest to the purely biological disciplines which study methods and conditions for creating artificial plant, animal and microbial communities and together with them works out the biocenology of closed systems.

In modeling a natural cycle of matter on a small scale, the essence of the processes in the new conditions of an artificial biosphere of a spacecraft remains unchanged in principle. However, the conditions under which these processes occur, the number of stages and functional elements involved and the spatial-temporal relations change so greatly that the model to be created will differ considerably from the original, primarily in such important parameters as the specific capacity of the system and the specific mass of the circulating substances and resultant rate of circulation and, consequently, in the reliability and viability of the system as a whole.

These are all common problems in modeling biological processes in integral systems. They may arise whatever the particular makeup of a system may be, and

they warrant special consideration. In addition, some special problems may arise in connection with the various physiological or biocenotic characteristics of concrete biological objects used in the system to realize certain stages in the circulation of substances. By way of illustration we should like to analyze one of the special problems pertaining to the biological regeneration of air in an inhabited spacecraft.

The activity of plants used for regenerating air is not always limited to the uptake of carbon dioxide and release of oxygen, as is the case with physicochemical methods of air regeneration. Systematic investigations in this field have just gotten under way, but the available data show that the atmosphere of confined spaces regenerated by means of unicellular algae and higher plants may be unsuitable. For example, plants are known to release volatile substances, essential oils, various hydrocarbons and other products of vital activity at different stages of their development. Carbon monoxide may be among these products (refs. 21 and 24). Other investigations of closed systems with air regenerated by unicellular algae have also demonstrated the possibility of carbon monoxide accumulating in concentrations ranging from 0.003-0.08 percent (refs. 11, 13, 16, 19, and 20). According to our own investigations (ref. 5), the concentration of carbon monoxide in reactors with the alga Chl. pyrenoidosa reached 0.007 percent and in most cases exceeded permissible concentrations for industrial quarters. In addition, there was an accumulation of hydrocarbons and sometimes of nitric oxide. /173

Similar complications may arise when any new functional link is added to a ecological system, because the vital activity of a given organism may result in the release of volatile and gaseous products. Unfortunately, our knowledge of this aspect of the activity of animals, plants and microorganisms is largely haphazard and therefore insufficient. There is no doubt that study of the extremely varied biochemical activity of living organisms in a closed ecological system will disclose new and especially toxicological problems for human ecology.

Thus, the successful solution of the problem of oxygen regeneration and carbon dioxide utilization by plants does not as yet answer the question of whether regenerated air can be used by man. This will require the introduction of new links to function as factors in the natural self-purification of the atmosphere now lacking in the existing systems.

The presence in the atmosphere of volatile admixtures released by plants, animals and microorganisms is also characteristic of natural conditions. However, the vastness of Earth's atmosphere and activity associated with the renewal of oxygen, which is believed to take about 2,000 years (ref. 8), must be taken into consideration. These 2,000 years are also a time when the released admixtures are exposed to a powerful array of factors that help to purify the atmosphere, e.g., light, ultraviolet and ionizing radiation, electrical processes in the atmosphere, precipitation, the tremendous sorption capacity of the soil and the entire surface and contents of the hydrosphere, which serve as physical or chemical solvents of volatile substances. /174

In an artificial low-capacity system, the duration of the period in which oxygen circulates is just a few days. This results in extremely rapid circulation of the regenerated components of the atmosphere and, consequently, it makes

unusually high demands on the mechanisms for purifying the air. These demands may not always be satisfied with the requisite completeness, economy or reliability. This suggests the use of great caution in setting up a plant community in an ecological system or, for that matter, in selecting any element of the system, from living organisms to building materials and roofing.

At first glance the simplest way may seem the most expedient--burning up all atmospheric impurities, which sometimes may be inevitable, despite the additional expenditure of energy required and the increased load placed on the system for removing heat from the craft. However, theoretically this is not the best way.

In creating an artificial ecological complex, it would be most efficient if it were fully capable of functioning as a naturally balanced, durably operating integral system that requires a minimum of artificial correctives. Instead of photosynthesizing organisms, it might be well to include plant forms which have the same main characteristics, but do the least damage to the habitability of the surroundings by releasing byproducts.

This is a highly promising approach, but it requires the planning and carrying out of extensive research of the quantitative and qualitative principles of metabolism in living organisms, on substances that have for the most part not yet been investigated. It is necessary simultaneously to investigate and to evaluate from the physiological-hygienic viewpoint permissible levels of concentration of these substances in the atmosphere of a spacecraft, both individually and in combination. The problem is further complicated by the fact that the biological significance for man of different atmospheric impurities varies, and some of the substances released by plants may also be beneficial, e.g., phytoncides.

The choice of optimum combinations of components of an ecological system from their effect on the composition of the atmosphere need not be solely empirical. Some common principles may be found for this purpose, e.g., the presence of a definite relationship between the phylogenetic age of /175
plants and the quantity of stable organic compounds which resulted from subsequent metabolism and accumulated in the plants' organs (ref. 1).

It may also be possible to use other biological objects that form the biocenosis of a spacecraft to absorb atmospheric impurities. It has been found that carbon monoxide can be reduced to carbon dioxide by some unicellular algae (ref. 15), and that soil bacteria can take up methane, acetone, alcohols and other volatile substances (ref. 9).

If we proceed from the fact that the air of a closed ecological system will contain admittedly larger quantities of impurities than the terrestrial atmosphere, due to the limited space, variety of influences affecting it and absence of factors for natural purification, the final choice of forms of organisms that least affect the normal composition of the atmosphere can be made only after taking into account the requirements of the human organism and the entire biocenosis of the spacecraft as a system that reacts integrally to various changes in the environment. In general, the habitability of the artificial atmosphere in a closed system ceases to be an independent problem when it is a question of

a complex of still uninvestigated substances that become a constantly acting factor in the environment of man and other organisms.

Water involves just as complex relations as air. This shows how interdependent the processes in the ecological system are, how closely human ecology is entwined with the ecology of other organisms, all of which constitute a single ecology of a community of living organisms and environment in a system with the recirculation of the substances.

In closed systems, the object of study, action and control is the metabolism and cycle of all substances circulating in all the links of the system. Even slight interference with the circulation of any of the elements that inevitably accumulate with time may result in disruption of the system. The matter of trace elements is worth noting in this connection. It requires the organization of a delicately balanced or controlled cycle in accordance with the requirements of each link in the system.

There are two important aspects of the matter which have a bearing on human ecology. First, the marked limitation of the population of the community responsible for the food requirements of man in the closed system and the repeated use of regenerated water may not satisfy the already known trace element requirements of the organism. Second, our knowledge of the trace element /176 balance in the organism is probably still insufficient, and the very first prolonged experiments in models of a closed system may introduce significant corrections into existing ideas. These considerations are not applicable, of course, solely to the human organism.

Both aspects require prompt solution, especially direct experiments simulating concrete systems with the recirculation of substances. Yet, the trace element problem has not been accorded the attention it deserves. The reason may be the seemingly easy solution of placing adequate amounts of the trace elements on board the craft. This would be a serious mistake. The viability of a closed ecological system is as dependent on a balance of trace elements as it is on a balance of all other substances. Thus, isolated consideration of the balance of certain substances purely on the grounds that the need for them is measured in microamounts is quite unsound.

It is quite obvious that the creation of reserves of trace elements will not by itself solve the problem of their balance in living organisms. The existence of such reserves presupposes the ability to use them. This requires a careful quantitative analysis of the actual trace element requirements of various organisms under specific environmental conditions. The matter is complicated by the fact that our knowledge of the physiological role of trace elements, their metabolism and balance in animals and plants is largely new and not completely worked out, specifically for systems with recirculation of substances. Continued ignoring of the trace element problem may convert it into a factor capable of limiting the practical realization of operating models of such systems.

The most important feature of human ecology in closed ecological systems is the considerable broadening of the man-environment relations with which it

is concerned. A broad ecological approach to evaluation of the biological acceptability of an artificial habitat for man will inevitably lead to the need of studying and normalizing those fundamental conditions of man that were never before the object of hygienic normalization--intensity and composition of optical radiation of the Sun, value of gravity, duration of the daily rhythm of vital activity. The rhythm of biological processes as an inseparable form of their manifestation in time, it seems to us, deserves special attention.

The many investigations of the daily and seasonal rhythms were aimed principally at studying their concrete manifestations and relationship /177
to cyclical changes under actual natural conditions within the framework of the terrestrial duration of the cycles (refs. 2, 6 and 7). The general biological nature and significance of the cycles themselves and their duration were not critically analyzed. The reason probably was that the existence of a daily rhythm of natural conditions and its particular duration are as immutable a constant for Earth as, for example, the force of gravity or magnetic intensity.

It was not until very recently that due to manned spaceflights the question arose of possible change in the conditions of the daily rhythms in human activity. It is possible to alter the daily rhythm of work of the spacecraft crew by modifying the content and volume of work called for by the flight plan (ref. 22). This matter was the object of an experimental investigation in which the 8-hour rhythm of activity of the subjects was studied and data obtained on the considerable degree of adaptability of the human organism to a change in the daily rhythm (ref. 17).

The absence of a natural daily cyclicity in the external conditions of spaceflight poses the question of whether it is necessary to create an artificial daily rhythm of vital environmental conditions for living organisms. Two main problems have to be solved in this connection--the duration of the "space" day and selection of the ambient factors that have to be artificially cycled to maintain the internal mechanisms that regulate the functioning of the organism.

The possibility of deliberately changing the duration of the daily cycle of human activity probably cannot be examined without analyzing the activity of the organism during spaceflight. For example, simple lengthening of the daily cycle with ordinary activity would merely mean increasing the daily functional load on the human organism and constitute an ordinary aspect of work hygiene.

Undoubtedly, it is not only changes in the current values of the physical environmental factors or the characteristics of their time sequence in the daily cycle that take part in the formation of the internal, endogenous mechanisms regulating the rhythmic activity of living substances, but also information about the content, material-energy relations and certain internal aftereffects of the organism's activity during each phase of the daily cycle. This information may be based on consumption of the reserve of any substance, on accumulation of products, vital activity, shock absorption or change in reactivity of individual functional elements and regulatory systems in the organism.

There is reason to believe that during spaceflight involving weight- /178
lessness or low gravitation, the content of this information may be changed, if

only as a result of a decrease in the gravitation component in the overall balance of the daily load on the muscular system. Consequently, during spaceflight the ordinary value of the daily functional load may be associated with a smaller expenditure of energy, that is, with less cushioning of the mechanisms responsible for transforming the energy of food into the specific work of the organs.

Under these circumstances either a shortening of the rest period or a lengthening of the daily cycle as a whole would be acceptable. The latter is preferable because it would preserve the familiar ratio of work to rest periods. We would obtain, as a result, a longer flight day while preserving the ordinary terrestrial ratio of sleep to wakeful periods and the values of energy consumption. In other words, we would have the functional equivalent of the usual rhythm of human activity, except that it would be longer from the standpoint of terrestrial time.

A favorable consequence of this lengthening of the daily rhythm might be expected as a result of the visible and objectively perceived (by the crew) shortening of the flight (reduction in number of flying days). Thus, lengthening the "space" day to, for example, 28 hours would create the feeling of a "4-year" flight, when the absolute duration was 5 years.

It is reasonable to assume that the subjective aspect of perceiving lengthened "space" days as ordinary terrestrial ones might also have an objective basis, if the energy characteristics of the organism's activity during terrestrial and flight days were equated. In other words, the "equal-energy qualities" of lengthened space and ordinary terrestrial days might be the objective basis for considering them physiologically and biologically equivalent.

The foregoing may give rise to the question of the general biological significance of the daily rhythm of vital activity as a form of "quantized" consumption of the individual stock of activity and lead one to assume the possibility (under certain conditions) of changing the absolute duration of the individual life cycle of the organism by changing the duration of the elementary (daily) cycles of its activity. Although it may appear to be worthwhile to lengthen the daily cycle for man, it would be desirable to shorten the day for the productive part of the animal-plant community as a possible means of shortening the processes of growth and development of organisms in absolute time and, consequently, increasing their biological productivity. The experimental studies under way in this field are expected to yield positive results. /179

In this article we examined only in preliminary fashion some of the problems that arise when man is included in a closed system with recirculation of substances. They show the extreme complexity of the physical relations of man in a closed system and the shortcomings of the traditional physiological, hygienic, toxicological and other approaches to the study of these relations.

The examples we cited also show that human ecology in closed systems can be successfully developed only as a general biological discipline, in close association with the ecology of other components of the system, only as one of the branches of the biogeocenology of a community of living organisms constituting a system with recirculation of substances under the specific conditions of a spacecraft cabin.

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HYGIENIC INVESTIGATIONS OF COSMONAUT CLOTHING DESIGNED FOR WEAR IN SMALL-SIZED CABIN UNDER SHIRT-SLEEVE MICROCLIMATE CONDITIONS

I. G. Popov, V. I. Krichagin, V. V. Borshchenko and F. K. Savinich

As flights grow longer, living conditions on spacecraft will become /180
increasingly important in maintaining the crew's health and a high level of working capacity.

Among other life-support factors involved in flight, the clothing of cosmonauts demands serious attention.

Spacesuits, which are designed to protect cosmonauts in emergencies from the extremely unfavorable aspects of the space environment, from abrupt changes in the cabin's microclimate, in landing, etc., do not provide adequately comfortable conditions for long wear. Experiments with modern spacesuits have shown that they are so awkward that they cannot be worn for long periods of time.

Continuing improvements made in the reliability of spacecraft cabins has indicated the feasibility of employing light and comfortable clothing with high hygienic characteristics for daily wear in flights.

The time is not yet at hand for the design of universal clothing for cosmonauts. It must be modified to suit a cabin's microclimate, the character of operations, flight duration, sanitary facilities, and other factors. How- /181
ever, one basic hygienic requirement may not be changed, namely, that the clothing must contribute to the utmost well-being and efficiency of the cosmonauts during flight.

By studying the hygienic aspects of cosmonauts' clothing for the purpose of establishing protective characteristics against heat, we finally chose a microclimate from among the possible temperatures found in aircraft cabins which would be considered comfortable for a lightly dressed person. The human thermal balance in this case could be maintained with minimal effort of the thermoregulatory processes. Perspiration, which can clog up the skin, thereby increasing the frequency of hygienically treating it, and the clothing, thereby impairing the latter's physicomechanical and hygienic qualities, must therefore be minimal. When at rest, most of the body exudes moisture due to imperceptible perspiration and only a number of areas excrete continuously "emotional perspiration." It is thus possible to introduce an efficient regime for the functioning of the skin glands, which would directly affect mineral and water

metabolism, the system for regenerating the gaseous environment of the cabin, sanitary facilities and the clothing itself.

Given a comfortable temperature, the clothing should be made of light, air-permeable materials possessing maximum hygienic qualities. It should also have thermal protective characteristics corresponding approximately to 1C10.

During spaceflight serious difficulties may be encountered in carrying out frequent hygienic treatment of the skin, frequent change of underwear and the entire attire. It would be impractical to carry a large supply of clothing because of weight; yet frequent cleaning of clothing is also difficult. Therefore, in sharp contrast to terrestrial conditions, the ability of clothing to cleanse the skin is a very important consideration. This function is ordinarily performed by underwear. From this stems the practical importance of investigating the possibility of designing clothing capable of prolonged continuous wear without hygienically treating most of the body.

Prevention of contamination of the skin and clothing in spacecraft cabins is facilitated by the virtual absence of exogenous contamination from different forms of dust. Skin and clothing are subject to contamination primarily by products of the body's biological functions (secretions of the skin glands, peeling epidermis, falling hair and particles of urine and feces).

Finally, the clothing of cosmonauts should be light, durable and sufficiently comfortable for the different kinds of activity associated with spaceflight.

Study of the literature (refs. 1-3) and laboratory testing of different fabrics resulted in the selection of promising knitted fabrics.

Knitted underwear and outer clothing, because of their porous mesh /182
weave, are characterized by softness, elasticity, wrinkle resistance, high degree of air permeability and considerable capacity to absorb moisture.

Knitted fabric can be made into snug form-fitting underwear and, what is particularly important for clothing to be worn under a spacesuit, it permits several layers of clothing to be combined and thus reduces bulkiness. Physiological sensors can easily be attached to it. Inasmuch as the prolonged wearing of a spacesuit is very uncomfortable, it is desirable that, together with improving clothing to be worn continuously, work should be done to design clothing intended for extended flights without a spacesuit.

For the purpose of selecting a second layer of clothing, a series of experiments was performed to study the effect of the nature of the fiber, air permeability and moisture characteristics of the outer clothing on the microclimate of the space under the clothing. These and other investigations resulted in the selection of a sports type of outer clothing made of knitted fabric for use under comfortable conditions.

An experimental model of footwear consisted of slippers made of chamois, according to our specifications, by L. V. Kedrov and his colleagues at the Scientific Research Institute of the Leather and Footwear Industry.

Physiological and hygienic evaluation of experimental models of cosmonaut clothing was made to determine a hygienically optimal version. It included the following main stages of investigation.

I. In a heat chamber:

(a) study of the effect of clothing models on heat exchange for the purpose of selecting a model which would best contribute to maintenance of the heat balance of the cosmonaut;

(b) study of the capacity of clothing material to absorb perspiration and other products of human biological functions;

(c) study of the irritating effect of the clothing on the skin.

II. In a mockup of a "Vostok" spacecraft:

(a) study of heat-protecting properties, perspiration absorption capacity and evaluation of the advantages of the clothing design through experimental wear in a spacesuit.

III. In flights on "Vostok" spacecraft.

A practical evaluation of optimal hygienic qualities of clothing models is presented.

Since present-day textile materials present no difficulty in designing clothing with the necessary heat-protecting properties for use at comfortable temperatures, special attention was directed to the study of contamination of the skin and clothing layers by products of the body's biological functions. Although this problem is important in connection with prolonged wear in flight, where there is no hygienic treatment of the skin, it has been little studied. Previously, the methods used (ref. 4) were devised for investigation of contamination under ordinary living conditions. They embraced isolated portions of the skin. Thus they could not be used to determine the general contamination levels of the body and the clothing. For this reason, I. G. Popov and F. K. Savinich devised a method suitable for the quantitative evaluation of the contamination of human skin and clothing by products of the body's vital functions. /183

This method was aimed at evaluating the following factors:

(1) degree of skin contamination by products of biological activity for the period that the tested clothing was worn;

(2) capacity of the underwear and other parts of the clothing to absorb products of biological activity from the skin surface;

(3) distribution of absorbed products of biological activity between parts and layers of the clothing;

(4) accumulation of chlorides and organic matter on the skin and in layers of clothing during the experiment;

(5) rate of perspiration during the experiment and indirect evaluation of the stress of thermoregulation.

Chlorides were used as indices of skin and clothing contamination by perspiration while the oxidizability of washes (in mg of O_2) was used as an index of contamination of the organism by organic substances.

The accumulation of chlorides and organic substances readily soluble in water on the skin and in the clothing was determined by chemical analysis of water washes from the whole body surface (excluding the head) and, separately, from the clothing. The chlorides were determined quantitatively in mg of Cl by Moore's method and the oxidizability in mg of O_2 by Kubel's method.

To prepare the skin before the experiment, the subjects thoroughly washed themselves under a shower.

Control washes were obtained by means of a bath from the clean skin as well as from the skin after the experiment. The bathtub was always filled with a carefully determined amount of water (120 liters) at constant temperature of 33-34°C, which would not intensify perspiration for a period of 15-20 minutes. From the bath, which had been filled with clean tap water, 0.5 liter was taken for background analysis for chlorides and oxidizability.

To obtain a skin wash, the subject immersed himself in the bath up to his chin for 5-8 minutes, all the while rubbing the body lightly with his hands. Special experiments have shown that such exposure is adequate to remove most of the soluble substances found on the skin. At the expiration of this period of time, another 0.5 liter water sample was taken for analysis. Following the removal of the control wash water, the subject got out of the bathtub, dried himself, and then put on the clothing under study. This was not taken off until the experiment was finished. Following removal of the wash water on the termination of the experimental wearing of the clothing, the subject washed /184 himself with soap and then put on his regular clothing.

Water samples from the bath before and after the subject was in it were investigated for chlorides (by Moore's method) and oxidizability (by Kubel's method). On the basis of background analyses of tapwater, a correction was introduced for the presence of these substances therein. The resultant data provide some idea of the amount of chlorides and organic matter on the subject's skin before and after the experiment.

The underwear and outer clothing were prepared in the following manner. New underwear and clothing first had their factory finish removed; used clothing was carefully laundered and rinsed.

To determine the initial presence of chlorides and organic substances in the underwear and second layer of clothing, both were soaked for two hours in tapwater. The volume of water used was determined experimentally (9 liters for an undershirt, 6 liters for undertrousers, 3 liters for socks, 9 liters for a sports shirt and 9 liters for the trousers).

The wash water from the clothing was tested individually for chlorides and oxidizability by the same methods. A correction was always made for the tapwater. The underwear and clothing were dried, pressed and then used for experimental wear.

Washes were obtained from the underwear and clothing after they had been worn in the described manner. After the clothes had been soaked for two hours, the samples of the water were tested for chlorides and organic substances.

Contamination of the skin and clothing was relatively small and was determined to a considerable degree by individual peculiarities of perspiration. Variation of the microclimate within the comfort zone did not materially affect skin contamination. Subsequent increase in the ambient temperature markedly increased the contamination. /185

The tested samples of the materials possessed a high capacity for absorbing chlorides and organic substances (80-95 percent absorption of the total amount of chlorides excreted through the skin). This capacity was retained even for wear as long as 30 days (table 1).

TABLE 1. DISTRIBUTION OF CHLORIDES BETWEEN SKIN AND CLOTHING (IN %) FOLLOWING EXPERIMENTAL WEAR IN HEAT CHAMBER FOR 10 AND 30 DAYS.

Knitted underwear*	Skin		Clothing	
	10 days	30 days	10 days	30 days
Cotton-rayon No. 1 ("smooth" fabric)	7.3	7.6	92.7	92.4
Cotton-rayon No. 2 (double-lasting "smooth" fabric)	5.7	6.0	94.3	94.0
Rayon fabric	7.8	5.2	92.2	94.8
Pure wool	12.6	7.2	87.4	92.8

*The outer clothing was standard, made of knitted cotton.

Similar relations were noted when investigating the distribution of organic substances (according to the oxidizability of washes in mg of O_2).

Moreover, to compare the ability of different fabrics to absorb biological waste products on one individual, clothing was issued which consisted of two parts sewn together from different materials. The results are shown in table 2.

The data in table 2 show that of the materials tested cotton-rayon fabric No. 2 (75 percent cotton and 25 percent rayon) manifested the highest ability to absorb chlorides and organic substances, while the wool fabric showed the lowest.

Investigation of the heat-protective and other properties of experimental samples of clothing was carried out in thermal pressure chambers and in mockups of the "Vostok" at temperatures of +17, +21, +25, +35 and +50°C and relative humidity of 30-70 percent.

Four sets of underwear, including the two most promising (cotton-rayon No. 1 and No. 2), as well as rayon and wool, were tested at all given temperatures for their physiologic-hygienic value. A sports suit or a spacesuit was put on over the underwear. Eight experiments were conducted at +17°, 12 at +21°, 12 at 25°, 25 at 35° and 35 at 50°. /186

The following was established as a result of the tests carried out in the heat chamber.

1. In experiments conducted at +17°, all subjects felt chilled at first due to lowering of the skin temperature of the extremities, a high level of heat loss and uncomfortable sensations of heat.

2. In all experimental models of clothing, the subjects felt comfortably warm in a state of rest at air temperatures of +21° and +25°.

3. At +35°, all subjects regardless of the clothing they wore exhibited a marked strain in thermoregulation (rise in body and skin temperatures, uncomfortable heat sensations).

However, the character of the changes noted in the physiological indices indicated that compensatory mechanisms of the organism are capable of preventing general overheating.

At these temperatures, the subjective and objective data were most unfavorable in the persons who wore woolen underwear.

4. At an air temperature of +50° all subjects, regardless of clothes, developed initial symptoms of overheating during the two hours they spent in the heat chamber. Persons who wore wool had the most unfavorable physiologic-hygienic indices and heat sensations.

5. Woolen underwear may irritate the skin, especially at high ambient temperatures and increased perspiration.

TABLE 2. DISTRIBUTION OF CHLORIDES AND ORGANIC SUBSTANCES IN CLOTHING, EACH HALF OF WHICH WAS MADE OF DIFFERENT MATERIAL, AFTER 10-15 DAYS OF WEAR.

Substances studied	Number of experiments	Set of clothing					
		No. 1		No. 2		No. 3	
		Cotton-rayon cloth No. 2	Cotton-rayon cloth No. 1	Cotton-rayon cloth No. 2	Rayon cloth	Cotton-rayon cloth No. 2	Woolen cloth
Chlorides, %	3	58.9	41.1	54.5	45.5	56.5	43.8
Organic substances, %	3	53.6	46.4	51.5	48.5	62.4	37.6

For the purpose of roughly evaluating the design of the clothing and footwear under conditions of weightlessness, samples of the clothing were tested during brief periods of weightlessness in flights of a laboratory aircraft.

The underwear specially designed for use with spacesuits, including a slit for sanitary purposes, worked well in combination with either a spacesuit or outerclothing for extended flights.

The skin of the subjects was studied by clinical observation and investigation of its physiological functions (dermatological examination, questioning of the subjects, determination of dermographia, study of the dynamics of change in the microflora on the skin surface, etc.).

Throughout the 30 day experiment, the condition of the skin of the subjects not hygienically treated remained entirely satisfactory. However, /187 there were manifestations of hyperkeratosis and thin-layer sloughing of the skin of the feet. During the course of the experiment a number of the subjects developed sycosis, occasional furuncles, dermatitis and acne vulgaris.

Sycosis was observed on the face where the skin was rubbed by helmet phones and on the back and chest because of increased perspiration brought on by physical exercises. By the end of the experiment, sycosis and dermatitis developed on the skin of the buttocks and perineum.

One subject had acne vulgaris. Due to more severe experimental conditions, he developed a general neurotic reaction, which contributed to a hypersensitivity to previously used electrodes, resulting in an allergic dermatitis in the region of the chest and on the inner surface of the right forearm.

It is thus apparent that clothing worn on the skin for as long as 30 days without any hygienic treatment did not appreciably affect the skin. The observed disorders did not interfere with the efficiency of the subjects or prevent them from carrying out the programmed tests.

The knitted underwear selected on the basis of our tests and used by Yu. A. Gagarin, G. S. Titov, A. G. Nikolayev, P. R. Popovich, V. F. Bykovskiy and V. V. Tereshkova in the early spaceflights was thus found to possess the necessary satisfactory hygienic qualities required for use in spacesuits during flight.

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THEORETICAL BASIS FOR THE MICROATMOSPHERE OF INTERPLANETARY SPACECRAFT CABINS AND PROSPECTS OF USING HELIUM-OXYGEN MIXTURES FOR THIS PURPOSE

B. M. Savin

There is no doubt that problems of a biological character now play /188
a leading, if not decisive part in the general plan for conquering outer space. One of the key problems is that of habitability, a major aspect of which is the theoretical and experimental basis of the microatmosphere of spacecraft cabins.

The gaseous composition of the microatmosphere of a spacecraft cabin is not as simple a matter as it might appear at first glance. It is no accident, therefore, that it has received little attention in the scientific literature. Up to the present time, hardly any attempt has been made to list the requirements which must be satisfied by the atmosphere in a spacecraft cabin.

On the basis of an exhaustive theoretical study of this problem, we believe it desirable to break down these requirements into three independent groups.

The first group includes requirements of a physiologic-hygienic character; the second, requirements relating to flight safety and shortening the time of going through the airlock when the members of the crew emerge from the cabin; the third, requirements of a technical nature.

The basic requirements of the first group include the following:

1. The atmosphere of the cabin should contain, besides oxygen, an /189
inert gas or gases to dilute the oxygen and provide the necessary pressure.

2. The gas pressure should provide adequate internal tension for bodily tissues to ensure their elasticity and prevent the organic fluids from "boiling" (altitude emphysema).

3. The partial pressure of oxygen should not be such as to induce toxic effects. At the same time it should provide optimal conditions of gas exchange at both normal and maximum permissible energy expenditures.

4. The gas composition and resultant pressure should provide optimal conditions for the normal maintenance of the heat balance at varying degrees of heat production due either to change in conditions and/or means of heat loss, particularly in case of disruption of natural convection during partial or total weightlessness.

5. The water vapor content of the cabin atmosphere should not interfere with perspiration within the limits of permissible physiological norms or cause excessive loss of moisture by the organism.

6. The composition of the microatmosphere should not include any substances or products of their interaction that would be harmful to the organism or require a prolonged period of adaptation.

7. The electrical state of the gases should provide favorable conditions for normal aeroion exchange between the organism and its surrounding gaseous environment.

The second group of requirements relates to problems of flight safety during emergencies (breakdown of the cabin's airtightness or the occurrence of fire) and to the creation of highly favorable conditions for reducing the time of going through the airlocks when the members of the crew step out into space. From our point of view these requirements would include the following:

1. The inert gases that form part of the cabin's atmosphere should possess a minimal coefficient of solubility and remain dissolved in the bodily tissues even with maximal supersaturation of the organism.

2. The inert gases that form part of the microatmosphere (diluents) should have the least possible volumetric weight and viscosity.

3. The gases making up the atmosphere should not present an explosive mixture. Furthermore, the content and partial pressure of oxygen should not present a danger in terms of explosion or fire. /190

The third group of requirements include chiefly the following:

1. The inert gases that form part of the microatmosphere should contribute to a simplification of the design of the apparatus intended to maintain the prescribed parameters of the microclimate and ensure the more reliable functioning of this apparatus.

2. The inert gases should have a minimum specific gravity and not have to be stored in bulky containers.

3. The inert gases should help to ease the work involved in finding leaks in the cabin if the airtightness is impaired.

There is no doubt that the list of requirements for the third group can be considerably expanded. However, no particular need exists for presenting them in greater detail.

What is the outlook for utilizing air as a gaseous medium in a spacecraft cabin, since it serves as man's natural environmental gas mixture?

From the point of view of the enumerated requirements, air for such a purpose is hardly ideal. Almost 80 percent of air consists of nitrogen, a gas which has a rather high specific gravity. The use of this gas to dilute oxygen and the gas employed for securing the required barometric pressure would make the microatmosphere of the cabin quite "heavy."

Nitrogen is readily soluble in body fluids and tissues, especially fatty tissue, as a result of which it may, during drops of barometric pressure, form bubbles in the tissues, giving rise to decompression disorders. In this connection it should be emphasized that the formation of bubbles can occur as a result of an acute drop in barometric pressure in an emergency situation (destruction of airtightness through puncture of the cabin) or certain operative measures, such as opening the hatch to let crew members of the spacecraft exit into space.

Although nitrogen is a relatively inactive gas, nonetheless, under certain conditions (when acted on by ultraviolet or ionizing radiation), it may take part rather easily in chemical reactions and thereby form toxic substances.

Atmospheric nitrogen is therefore hardly desirable as a component for the microatmosphere of a spacecraft cabin because of its specific gravity, sol- /191
ubility in biological substrates, capacity for forming toxic compounds and for other reasons.

However, as has already been pointed out, the microatmosphere of a spacecraft cabin cannot consist of oxygen alone. It must include gases to create the necessary pressure and to dilute the oxygen. Recent years have seen the accumulation of a mass of experimental findings supporting the view that prolonged exposure of man or animals to an atmosphere consisting solely of oxygen even at ordinary partial pressures is injurious (ref. 1).

The theoretical basis of the gaseous composition of the microatmosphere of a spacecraft cabin is without doubt a most complicated problem, calling for broad scientific research. It is perfectly obvious that prime attention should be directed first of all to those gases which, according to present-day knowledge, do not possess any marked biological action and yet fully satisfy the aforementioned requirements with respect to physicochemical properties.

In recent years, the foreign press has repeatedly raised the question of using helium because of its physicochemical properties (refs. 2 and 3). Helium has a number of advantages over nitrogen. Chemically, it is more inert and does not form any toxic compounds. Its solubility coefficient in biological media is not high. What is more important, helium is poorly soluble in fats; on desaturation it leaves the organism twice as fast as nitrogen. Thus, the use of helium may prevent decompression disorders induced by rapid drops in barometric pressure. For the same reasons, its inclusion in the microatmosphere should help reduce the time in going through the airlocks on emerging into space.

The low specific gravity of helium should promote a smaller energy expenditure when breathing and reduce the amount of energy consumed in circulating the gas mixture through the air conditioning system.

Finally, helium possesses much more heat conductivity than nitrogen. /192 This feature would be helpful in weightlessness and in the absence of convection.

The marked differences between helium and oxygen in thermal conductivity may be used as a physical parameter governing the operation of the apparatus responsible for keeping the gaseous composition of the microatmosphere constant. In view of the foregoing, helium, judging by its physicochemical properties, should be considered one of the best diluents of oxygen and components of the microatmosphere that maintain the necessary values of general barometric pressure in the spacecraft cabin.

However, the possible use of helium as a component of the microatmosphere of a spacecraft cabin has not been adequately investigated up to the present time. Despite the fact that several investigations (refs. 4-8) have established the possibility of man existing in a helium-oxygen atmosphere used as a means of preventing the narcotic action of nitrogen in deep-water diving operations, a number of authors continue to believe that prolonged exposure to helium at relatively high partial pressures of helium may adversely affect organic viability.

Actually, the effects of prolonged exposure to a gaseous environment containing high partial pressures of helium has been poorly studied up to the present time. Most experimental findings in this connection were obtained in investigations with partial pressures of helium considerably in excess of 1 atm. However, the duration of the organism's interaction with modified conditions of the gaseous environment was very brief in these experiments. For these reasons most of the information on the effect on the organism's physiological functions of replacing nitrogen with helium is only of indirect interest to space medicine.

It is no accident that Bartlet and Philips (ref. 9),in speaking of a microatmosphere lacking in nitrogen, emphasize that "the problem may be considered solved as far as brief exposure to such an atmosphere is concerned. But for long periods of time it is necessary to take into account the effect of the absence of nitrogen on heredity..., the reproductive capacity, and the processes of growth and development of the organism."

The reason for such fears is not only the absence of the necessary experimental data on the effect on the organism of the prolonged action of high partial pressures of helium, but also the absence of an answer to the question of /193 the so-called biological action of nitrogen. In the opinion of a number of authors, nitrogen is not an absolutely inert gas, because it has definite biological significance (refs. 10 and 11).

Certain authors concluded from their experiments that the normal functioning and development of an organism is absolutely impossible in an environment lacking in nitrogen (ref. 12). However, the results of these experiments have been severely criticized and not widely accepted. However, the absence of any experimental proofs to the contrary has caused investigators to be cautious. In

particular, inclusion of one-sixth part nitrogen in the atmosphere of a Manheim II balloon cabin was no accident and did not present any technical difficulties (ref. 13). The reason for this was the fear that total exclusion of nitrogen from the composition of the surrounding gaseous environment would have an adverse effect on the human organism.

The complexity of the problem is largely due to the fact that in spaceflights the organism will be exposed to a modified gaseous environment for many months and possibly years. Under such conditions, the time factor may play a decisive role, since relatively minor changes may lead in the course of time to qualitative changes absolutely undesirable for the human organism.

A number of experiments were carried out during 1960-1962 in order to clarify the matter. The experiments involved a broad study of problems relating to the biological significance of nitrogen and to the effect on the organism of prolonged exposure to a gaseous environment containing high partial pressures of helium. Some experiments were performed for the purpose of elucidating the influence of the physicochemical properties of helium on the course of fundamental physiological processes, specifically, gas exchange (external respiration function, gas exchange in the lungs), on cardiovascular activity, on the central nervous system (conditioned reflex activity), on energy expenditure, maintenance of the heat balance and other physiological indices.

In order to create the most favorable conditions for determining the biological importance of nitrogen and elucidate the effect of prolonged exposure to high partial pressures of helium, experiments were carried out on chick embryos and frog egg cells. The high intensity of the metabolic processes in the early stages of the development makes such objects particularly useful for the study of any kind of unfavorable environmental influences.

The research on chick embryos, which we conducted jointly with /194
V. V. Boriskin, P. A. Gul'tyayev, P. F. Oblapenko and V. V. Rol'nik, established that the exclusion of nitrogen from the surrounding gaseous environment and its replacement with helium had no unfavorable effect on the developing organism.

Experiments studying the development of frog egg cells (V. V. Boriskin, A. V. Voyno-Yasinetskiy and B. M. Savin) corroborated the conclusion that the influence of high partial pressures of helium is due purely to its physical properties (heat conductivity and capacity). There is no basis for thinking that helium has some specific effect on the organism.

Thus, the investigations conducted on chick embryos and frog egg cells completely refute the view that the organism cannot develop in an environment deficient in nitrogen and containing high partial pressures of some other inert gases. The substitution of helium for nitrogen in the surrounding atmosphere is no obstacle to the normal course of embryonic development.

Experiments conducted on chicks and mice (V. V. Boriskin and B. M. Savin) showed that prolonged exposure of animals to a helium-oxygen atmosphere does

not produce any disorders. The general activity of the animals, their food excitability and reaction to various stimuli during exposure to a helium-oxygen environment is much more dependent on the ambient temperature than on the atmosphere.

During prolonged exposure to a helium-oxygen environment, the zone of thermal comfort shifts significantly towards higher temperatures by about 3-3.5°. The cooling action of a helium-oxygen environment has been further corroborated by experiments on a dog. During the first few days of exposure to a helium-oxygen atmosphere, the animal exhibited definite changes in external respiration, in intensity of gas exchange and energy expenditure, cardiovascular function and thermotopography. However, when the temperature was raised to approximately 27° in the chamber, all changes previously observed in the dog disappeared completely, and no marked abnormalities were found in any of the organism's basic physiological functions. Consequently, the observed changes were due to increased heat emission by the organism into the environment.

The effect of substituting helium for nitrogen on gas-exchange processes in the lungs was studied by us jointly with Chang-Ch'un in experiments with dogs. These investigations confirmed the observations of a number of authors that /195 breathing of helium-oxygen mixtures causes a significant decrease in the fluctuation of intratracheal and intrapleural pressures. As a result, the expenditure of energy on external respiration decreases considerably. The view repeatedly expressed in the literature that helium exerts a favorable action on the diffusion of oxygen through the alveolar wall was found to be fallacious, as demonstrated by hypoxic helium-oxygen and nitrogen-oxygen mixtures.

Analysis of the experimental data has shown that the superior oxygenation of the blood during the breathing of helium-oxygen mixtures is due solely to the fact that the intensity of pulmonary ventilation is usually much greater than when breathing air because of the lesser density of these mixtures.

These experimental findings examined show that the exclusion of nitrogen from the surrounding gaseous environment and its replacement by helium does not produce any deleterious effect on the organism or cause marked changes in its physiological processes. However, the zone of temperature comfort is about 3-3.5° higher than that of ordinary air. We conclude, therefore, that helium can be used as a component of the microatmosphere of spacecraft cabins.

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CYBERNETICS AND SPACE BIOLOGY

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One of the special features associated with investigations in /196
space biology and medicine is that the investigator is situated at a considerable distance from the biological subject.

This situation has made it necessary to apply objective methods for recording and transmitting biomedical information. The problem was solved by developing telemetering systems which transform the diverse biomedical data into electrical signals and transmit them over a rather large distance. At the receiving points the information is again transformed, so that the investigator obtains it in the form in which it was measured on the biological test subject (refs. 1-3).

It has been possible to achieve this only when the characteristics of the biological subject can be represented by a large series of quantitative relationships.

The availability of substantial experimental data on the quantitative characteristics of the biological test processes makes possible their comprehensive analysis, including analysis performed with the aid of the exact sciences. In this respect, due to the characteristics of cybernetics, it finds an ever-increasing application in biology and medicine (refs. 4-6). However, the penetration of cybernetic methods into biological sciences and particularly into space biology and medicine will become even greater with time than it is today. The scientists who specialize in this field will have to become thoroughly familiar with what we might call "the cybernetic method of thinking." The special features of this method involve a more general approach to a problem and an effort to find an analogy between the biological /197
phenomenon and processes which take place in engineering devices, so that the latter can be used to make a more detailed investigation of the biological phenomenon.

With this approach to the investigation of biological subjects it becomes necessary for specialists in biology and medicine to apply the conventional methods of engineering science.

Some of these methods together with their most simple applications to space biology and medicine are considered below.

1. Mathematical Simulation

One of the trends in modern science is the wide application of mathematical methods to the most diverse fields of knowledge. The process involving the mathematical formulation of investigations in various branches of science is now quite firmly established, because at the present level of our knowledge it is no longer possible to limit ourselves to the qualitative description of observed phenomena and the qualitative formulation of the laws which govern their behavior. It is also necessary to study the quantitative laws governing the behavior of these phenomena.

Simulation applied to biology, as we know, is by no means an exhaustive approach to the investigation of the biological process itself, but it does help to study some of its features and to establish laws (even though they may be very approximate) which remain unobserved during conventional experiments (refs. 7-9).

Mathematical methods for investigating biological processes are applied more and more in space biology and medicine (refs. 10-14). In references 13 and 14, which are concerned with the investigation of the pulse activity of the atrium receptors and neurons of the giant-cell nucleus of the reticular formation, the author has made an attempt to produce a basic model of the otolith part of the vestibular apparatus.

The model which has been developed explains a series of functions of this important organ, and also explains some processes of information conversion in the neurons of the reticular formation.

One of the special features of the model developed for the otolith part of the vestibular apparatus is the presence of a "summation device" which compares the pairs of signals received from the receptors of the left and right utricle as well as from the saccule.

As shown by O. G. Gazenko and co-authors (ref. 14), the presence of this summation device may be used to explain the functions and operating principles of mechanisms which control the flight organs of birds, and also the nature of animal and bird motion when the otolith apparatus is partially removed /198 in these creatures.

Rather interesting relationships have been obtained in the investigation of the simulation function, when low frequency mechanical oscillations act on the organism and when the organism is subjected to weightlessness. During the rocking motion a periodic component appears at the output of the summation device. This component is due to the variability of the otolith apparatus receptor sensitivity during prolonged action of periodic oscillations on the organism. In the case of weightlessness the receptors of the otolith part will generate random pulses, because the membrane remains in a neutral state due to the absence of a gravity force. The intensity of impulsation apparently increases sharply when the head is moved, producing a certain component at the output of the summation device. Thus the processes in the basic model of the vestibular apparatus during rocking and weightlessness will take

place in approximately the same manner. We feel that further developments to improve the model for the otolith part of the vestibular apparatus will explain other functions performed by this organ.

It would be of considerable interest to design a model to explain the mechanism of the process which takes place in the human organism under the action of a radial G-force. We made an effort to apply simulation when we considered the question of the human endurance limits for these G-forces. In formulating this problem a working hypothesis was selected which assumes that the disorders in a human being caused by the action of radial G-forces are proportional to the square of their values, while the compensation reaction of the organism is proportional to the first power of the G-force. The deficiency which arises in this case and which is permitted by the organism exactly determines the permissible time for the action of a G-force on the human organism. This operating hypothesis was represented mathematically in the general form.

We found that the values of the permissible G-force action time, obtained by means of this equation, are in rather close agreement with the values of time obtained experimentally for the same values of the G-forces. Further work in this field may make it possible to design a model which will reflect this phenomenon more accurately. This is important in predicting the capacity of a subject to withstand a given G-force, based on his preliminary investigation.

2. Biological Control

It is known that during the entire period of vital activity a /199
series of quantities are controlled in the organism of animal and of man. Published literature shows that when a special experiment is conducted to cause artificial deviation of a control quantity in an organism from its normal value transient processes take place similar to those in engineering systems of automatic control.

G. Drishel' (ref. 15) carried out investigations on the change in the pulse rate during the so-called Shellong test, i.e., when a person is moved from a horizontal position to a vertical position. It was established that the curve showing the change in the pulse rate as a function of time may have a different form, beginning with a slow increase to its terminal value and ending with a rather rapid variation up to the terminal value. Under these conditions it is also possible to have damped oscillations.

It is of great interest to consider the degree of dynamic stability associated with the system for controlling the vegetative functions of man.

G. Drishel' analyzed a series of reports and came to the conclusion that the same type of basic control for specific vegetative functions is retained in a specific individual under different conditions and during a prolonged period of time, i.e., an aperiodic or damped oscillatory process takes place. During this time the variation in the magnitude and type of loading, the

state of the organism at a given instant of time and the action of various exogenous factors have almost no effect on the type of control inherent in a particular individual.

These special features, which are associated with the dynamic characteristics of the control system for man's vegetative functions, can also be used successfully in space biology and in medicine, when physiological investigations are carried out. Information on control characteristics is important in evaluating the stability of the human organism when the latter is considered as an automatic control system with various flight factors acting on it.

When a human organism is under the influence of external factors, its existing transition to a different level of functioning, even when the basic physiological functions do not exceed the permissible deviations, apparently cannot serve as a proof that the organism is indifferent to these external factors. The fact is that as a result of those unusual external factors most frequently investigated in space biology and medicine (apparently G. Drishel' did not consider this), definite displacements can take place in an organism, producing a change in the dynamic control characteristics of the vegetative functions. However, the presence of such changes cannot be /200 determined when the processes associated with the vital activity of the organism are investigated under static conditions.

The existence of deviations in the dynamic characteristics associated with the control of vegetative functions from their optimum setting may be the reason for functional disorders in an organism which occur only after the organism has existed under these specific conditions for a certain period of time.

The possibility of such disorders may be detected earlier by investigating the dynamic characteristics of the system which controls the vegetative functions in the organism of man, using the methods of automatic control theory (ref. 16). As an example of this approach to the investigation of physiological functions we consider the analysis of man's cardiovascular system reaction to mechanical effects experienced by the organism.

It is known that in space biology and medicine three types of mechanical effects on the human organism are studied--shock, radial and vibratory accelerations, which act on the organism of man as a whole. If the reaction of the cardiovascular system to such effects is described only by such integral indexes as pulse rate, it is possible to establish some analogy between the engineering systems of automatic control and the cardiovascular system of man. In evaluating the quality of automatic control systems, the method of typical perturbations is used. In this method a signal of definite form is fed to the input of the system, and the reaction of the system to this signal is investigated.

Typical perturbations usually consist of a rather short pulse, an effect which varies in steps, and harmonic oscillations. The mechanical effects used for investigations in space biology and medicine have a similar form (shock, radial and vibratory accelerations). The response of the cardiovascular system

to pulse and step effects has a great deal in common with the response of an automatic control system, as has been shown by experimental evidence.

The existing analogy between the response of a cardiovascular system of man to shock and radial accelerations and the response of an automatic control system to step functions requires that we clarify the reasons why there is no analogy between the response of this system to G-forces which vary harmonically. The mathematical analysis of existing experimental data and the analytical determination of frequency characteristics associated with the cardiovascular system have shown that harmonic G-forces with a frequency greater than 0.1 cps have no effect on the cardiovascular system of man from the standpoint of control. This characteristic of the cardiovascular system explains the absence of variations in the pulse rate, when the human organism is subjected to overall vibrations with a frequency of 10 - 70 cps. /201

It was established that the effect of periodically varying accelerations, with a frequency of 0.1 - 0.3 periods per minute, on the human organism is undesirable, because it can produce resonance phenomena in the system, which, in turn, leads to an increase in the rate of cardiac contractions. This increase is considerably greater than with constant value acceleration.

The curves showing the variation in the pulse rate when the organism is subjected to typical perturbations may be used to establish criteria for the quality of the cardiovascular system as an automatic control system. Apparently this will make it possible to develop a much more sensitive methodology for evaluating the displacements in the organism from different effects.

We utilized the integral evaluation of the control quality to establish the possible variations in the cardiovascular system control of V. F. Bykovskiy during his spaceflight, produced by the prolonged action of weightlessness and the limitation of mobility. For this purpose we processed the results of investigations on the reaction of his cardiovascular system accomplished by means of an orthostatic test. We found that when we evaluated the quality of this system control for three positions of the cosmonaut's body, the best quality of the cardiovascular system before the flight was achieved in the order of "standing up," "with the head down" and "lying down."

After the flight, the cardiovascular system control had the best quality when the cosmonaut was "lying down," then came "standing up" and finally "holding the head down." In addition, after the flight there was deterioration in the control quality in the "standing up" position and "with the head held down," and an improvement in the "lying down" position.

We believe that these data show the presence of definite displacements occurring in the organism and the direction of these displacements.

The examples which we have presented for the application of the methods of automatic control theory to the study of biological processes show that their application in space biology and medicine is quite promising.

3. Statistical Dynamics

During variation of such external factors as the surrounding temperature and pressure, radial G-forces, the organisms of higher animals and of man experience a rearrangement in the functioning level of organs and organism systems manifested by an increase in the rate of cardiac contractions, /202 respiration rate and arterial pressure. Since the values of these physiological indicators to some extent reflect the state of the test organism as a function of external factors, in the mathematical description of this state these indicators may be taken as the "output functions" of the organism. The "input functions" of the same organism in our case will be the external effects on the test organism (ref. 11).

It is of great interest to establish a mathematical relationship between the external effects and the reaction of the organism to these effects, since the availability of such a relationship can apparently be used for a certain prediction of the organism's state and is expected to give a clearer understanding of the experimental processes occurring in the organism itself. However, the mathematical analysis of biological processes is associated with a series of difficulties, because biological dynamic systems, from the point of view of cybernetics, represent systems with statistically determined parameters. The latter situation, from the same point of view, is the reason for the presence of an element of chance when determining a specific value for the indexes of the organism's "output functions." Therefore, in the general case when a mathematical description is given of the indexes defining the physiological functions of an organism, it is apparently possible to use the analytical methods developed for the statistical analysis of automatic control systems.

The methods of statistical dynamics, developed in the works of V. S. Pugachev, V. V. Solodovnikov, I. Ye. Kazakov, B. G. Dostupov and others (refs. 16-19), provide for a series of statistical characteristics which can be determined both for the external effect as well as for the subject itself. Such characteristics are usually the mathematical expectation, dispersion, correlation function, mutual correlation function and a series of other quantities.

The utilization of the methods of statistical dynamics assumes a wide application of computer technology, since a large number of calculations are required to determine these statistical characteristics. The difficulties associated with developments in this direction will unquestionably be paid for by a series of substantial advantages to the experimenter using the methods of statistical dynamics in his work.

The application of these methods could result in the development of comprehensive techniques for investigating biological objects. Such techniques would consider the mutual relationships between organs and systems.

It is expected that this approach will extract additional medical information from the accumulated experimental data.

The analysis of existing data shows that in principle it is /203
possible to obtain such information as illustrated by the example presented below.

The R-R intervals of the electrocardiogram are usually used to determine the rate of cardiac contractions, in accordance with a well established methodology. However, if the same data are used to compute the so-called normalized autocorrelation function, the periodic component of the latter, which is detected in this fashion, determines, as we found, the respiration rate of the subject at the time of recording the electrocardiogram. This curve was found by the mathematical processing of the electrocardiogram R-R interval because there is some coupling between the cardiovascular system and the respiratory system. This coupling is manifested in the form of the respiratory arrhythmia.

The use in space biology and medicine of the general principles of analyzing complex systems developed by statistical dynamics will apparently make it possible to improve the methods of investigating biological processes and to develop more refined methods of their analysis. In particular, we can assume that these methods will be used to carry out quantitative analysis of effects produced by the action of internal factors on an organism when the variations in physiological function indicators are exhibited only weakly.

It is known that such flight factors as G-forces, elevated temperature of the surrounding air and others, varying within the limits encountered under true flight conditions, produce such a strong effect on the organism that they appear as variations in the "output functions" of the organism (rate of cardiac contractions, respiration rate, arterial pressure, etc.), with respect to their initial values. In this case the investigation of dynamic processes occurring in an organism may be accomplished by using the methods of automatic control theory, whose simplest applications have been considered here.

However, in analyzing the nature of the organism's reaction to certain effects, the experimentator frequently encounters insignificant variations in physiological function indicators. In a series of cases it is practically impossible to distinguish between the reaction of an organism to the test effect and the normal variations of physiological function indicators present in the organism when at rest.

From the point of view of statistical dynamics this problem is quite similar to the well-known problem of isolating a weak signal in the presence of noise. Indeed, in any biological experiment the experimentator tries to detect variations in behavior, reactions and in the processes of vital activity of the test organism produced by the factor under investigation, /204
which generally can be looked upon as "signals." The normal reactions of the organism, the measured physiological function indexes which are established before the effect of the test factor, will represent certain "disturbances." Therefore, the minor variations in physiological function indicators produced by the test effect, which the experimentator is trying to isolate, will in our case represent the unknown "useful signal."

The experience of applying this method was verified during the study of the effect of prolonged restriction of animal (dog) movement on the functioning level of the cardiovascular system. It was necessary to use this approach to the analysis of existing experimental data, because, in considering the recordings of the animal's pulse rate when the animal was under conditions of restricted movement for a long period of time, it was impossible to draw any conclusions on the nature of the effect produced by this factor on the animal organism, due to its large variability. However, when a comparison of cardiac contraction at different periods of the experiment was replaced with a comparison of the statistical characteristic of this quantity in the form of mathematical expectation, it became possible to isolate the presence and direction of displacements occurring in the cardiovascular system of the animal under investigation.

Since in this example the application of the simplest analysis usually carried out in statistical dynamics gave a positive result, this indicates, we believe, that such an approach to the analysis of biological processes is promising.

We have considered only a specific case for the application of the methods of biological cybernetics to physiological investigations. However, the material presented undoubtedly points to the extreme importance and expediency of applying these methods to the problems of space biology and medicine.

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APPLICATION OF SOME OF THE CONCEPTS OF INFORMATION THEORY TO THE ANALYSIS OF PHYSIOLOGICAL DATA OBTAINED DURING SPACEFLIGHTS

A. D. Yegorov

At the present time the analysis of physiological data is qualitative in nature and is presented in a descriptive manner. /206 There is no doubt that such evaluation of experimental data contains elements of subjectivism.

The probability-statistical methods of analyzing physiological data are not widely used. The theory of information, which is a branch of probability theory, has found no practical application in the analysis of physiological data. There is only one reference on this subject (ref. 1). In the present report an effort is made to apply the ideas and methods of information theory to the analysis of physiological data obtained during spaceflights. The basic concept in information theory is the concept of entropy. Let us examine this concept with a view of utilizing it for the analysis of physiological data.

By recording physiological indicators we obtain certain information on some organism's system, whose state at each instant of time is random.

It is a trivial conclusion that the recording of physiological indicators is meaningful, because we do not know a priori the state of the test organism's system and consequently do not know the indicators which reflect its state. In the contrary case any physiological investigation would be devoid of meaning. In other words, each physiological system of the organism at the time of investigation may be in some random state unknown beforehand. This means that the physiological systems of the organism contain some degree of indeterminacy: the less we know about the functional state of a physiological system /207 before the investigation, the higher is its indeterminacy and, consequently, the more valuable will be the information obtained during investigation. As an example we consider data on the cardiac contraction rate and respiration rate obtained during multiple repeated recordings using a healthy young person under identical conditions (table 1).

From the data presented it follows that the pulse rate varies from 63 to 78. This means that when we start our recording we cannot predict what the pulse rate will be.

The question arises as to how the indeterminacy can be described quantitatively and on what it depends. With this purpose in mind we shall consider data on the distribution of cardiac contraction rates shown in table 2.

TABLE 1. VARIATIONS IN CARDIAC CONTRACTION RATE AND RESPIRATION RATE IN A HEALTHY PERSON DURING REPEATED RECORDINGS UNDER IDENTICAL CONDITIONS.

No. of measurement	Pulse rate	Respiration rate	No. of measurement	Pulse rate	Respiration rate	No. of measurement	Pulse rate	Respiration rate	No. of measurement	Pulse rate	Respiration rate
1	64	11	14	73	13	26	68	9	39	74	12
2	68	13	15	75	18	27	62	10	40	75	10
3	66	12	16	75	16	28	68	14	41	78	14
4	64	15	17	76	14	29	69	13	42	72	10
5	66	18	18	72	15	30	70	15	43	72	14
6	68	14	19	78	11	31	70	16	44	70	13
7	69	14	20	74	16	32	69	14	45	72	12
8	66	17	21	74	14	33	70	16	46	74	11
9	68	16	22	64	12	34	70	18	47	74	12
10	76	16	23	63	10	35	68	16	48	70	15
11	70	14	24	68	12	36	68	17	49	65	14
12	74	16	25	66	8	37	69	16	50	72	14
13	70	17				38	74	18			

TABLE 2. DISTRIBUTION OF PULSE RATE OBTAINED DURING SUCCESSIVE RECORDING OF SUBJECT K UNDER IDENTICAL CONDITIONS WITH INTERVAL = 5.

Indicator	Interval			
	61-65	66-70	71-75	76-80
No. of cases	6	24	16	4
Probability	0.12	0.48	0.32	0.08

Table 2 contains several columns showing the intervals, the number of times when certain values of the pulse rate occur and the respective probabilities.

It is quite clear that the larger the intervals, the higher is the degree of indeterminacy. However, the degree of indeterminacy depends also on how frequently the considered indicator lies within the limits of some interval, i.e., on the probability of assumed values. Indeed, if we conditionally divide the values into two groups, such that the first group contains /208 the readings when the pulse rate is up to 77 times per minute (48 cases), while the second group is for pulses above 77 beats per minute (2 cases), then

in proceeding with the investigations, we can state with almost complete assurance that the pulse rate will be in the range 62-77. In this case indeterminacy is negligible.

If the division, on the other hand, is made in two groups, such that the number of cases is approximately the same (in the first group 62-69 beats per minute--22 cases, in the second group 70-78 beats per minute--28 cases), then it becomes more difficult to predict in which group the pulse rate will fall in the subsequent recording. It is even more difficult to do this if there are more than two groups and if the number of cases in each of these is approximately the same.

Thus the degree of indeterminacy depends on the number of possible intervals within which the physiological indicators can lie and on the probability with which they appear in any of these intervals.

In information theory the measure of indeterminacy is the entropy (ref. 2)

$$H(X) = \sum_{i=1}^{n} Pi \log_2 Pi,$$

where H(X) is the entropy, P_i is the probability of random quantity values.

In the example cited the magnitude of the entropy for the pulse rate (table 2) is equal to 1.6929 binary units: [H(PR) = -(0.12 $\log_2$ 0.12 + 0.48 $\log_2$ 0.48 + 0.38 $\log_2$ 0.32 + 0.08 $\log_2$ 0.008) = 1.6929 binary units].

It is obvious that if we decrease the value of the intervals and thereby increase the number of possible states which can be assumed by the test quantity, the magnitude of entropy will increase. Thus, in the preceding example if we /209 make the interval equal to 1 beat per minute, the entropy will be equal to 3.4685 (table 3).

Obviously the values of entropy for the physiological indicators can be compared only when the intervals are of equal value.

During the continuous decrease in the intervals the entropy increases, and when $\Delta X \to 0$ (ΔX is the value of the interval) the entropy approaches the value (ref. 3)

$$\underset{\Delta x \to 0}{H(X)} = -\int_{-\infty}^{\infty} f(X) \log f(X)\, dX - \log \Delta X.$$

In this expression when $\Delta X \to 0$, the quantity $-\log \Delta X$ increases without limit ($-\log \Delta X \to \infty$), while the first term $\int_{-\infty}^{\infty} f(x) \log f(x)\, dx$ does not depend on ΔX, where f(x) is the distribution density of the random quantity X.

TABLE 3. DISTRIBUTION OF PULSE RATE OBTAINED WITH SUCCESSIVE RECORDING OF SUBJECT K UNDER IDENTICAL CONDITIONS WHEN INTERVAL = 1.

Indicator	Interval								
	62	63	64	65	66	67	68	69	70
No. of cases	1	1	3	1	4	0	8	4	8
Probability	0.02	0.02	0.06	0.02	0.03	0	0.16	0.03	0.16
	Interval								
	71	72	73	74	75	76	77	78	
No. of cases	0	5	1	7	3	2	0	2	
Probability	0	0.10	0.02	0.14	0.06	0.02	0	0.02	

Note. H(PR) = 3.4685 binary units.

Consequently, if we know the distribution law for the continuous random quantity the entropy can be computed by means of this equation. In particular, when the random quantity (ref. 4) has a normal distribution law: $H(X) = \log \left[\frac{\sqrt{2\pi e}\cdot\sigma}{\Delta X}\right]$ (σ is the root-mean-square deviation of the quantity X).

In practice this considerably simplifies the calculation of entropy.

Entropy, therefore, is a quantitative measure of indeterminacy. When /210 information obtained during spaceflight is processed, it is expedient to compute the entropy together with other statistical parameters. During the physiological analysis this entropy can be used to establish the quality of control exercised by the test system of the organism.

Let us consider this question in more detail.

It is known that the activity of sensitive cells of the highly differentiated organism of man or of animal takes place normally only if we provide for constant conditions in the medium surrounding the cell.

These constant conditions are provided by the neurohumoral mechanisms (refs. 5 and 6). In the opinion of Drishel' (ref. 7), there is some analogy between the neurohumoral regulation and the operation of a technical control system. In both cases the essence of control is reduced to the maintenance of the controlled quantity at a preassigned level. If the controlled quantity did not deviate from a given level, it would have no indeterminacy, i.e., its entropy would be equal to zero.

However, the organism of man is continuously subjected to external and internal perturbations deflecting the controlled quantity from its state of equilibrium. The displacements resulting from this produce a flow of information into the control centers providing for correction of the efferent impulsation.

Thus, at each instant of time the control quantity may assume any random value which is not known beforehand, i.e., it will be characterized by a certain indeterminacy.

Obviously the better the control, the less will be the indeterminacy of the controlled quantity.

Within the limits of a recording session the conditions of the cosmonaut during a period of one recording cycle remain almost unchanged. Therefore, the indeterminacy of the investigated random sequence of physiological indicators will depend on how well regulation is achieved under given environmental conditions and on the degree to which the adaptive reactions are developed.

As an example we cite data on the value of entropy for the rate of cardiac contractions of G. S. Titov during orbital flight. During the first loop, H(PR) was 4.97 binary units, while during the sixth loop when the vestibulovegetative disorders reached a maximum value, it increased up to 5.61 binary units. However, the average rate of cardiac contractions during the 6th loop was substantially less than at the beginning of flight. (During the first loop it was 101 and during the 6th it was 89 beats per minute.)

This indicates that the physiological interpretation of entropy /211
as a measure of indeterminacy and of dispersion as a measure of unsteadiness is very similar. In the quantitative relationship the magnitude of entropy for the distribution of the investigated sequence of indicators according to the normal law is a function of dispersion.

However, the concept of entropy is more general. On the one hand, entropy may be expressed in definite dimensionless units with values depending only on the base of the logarithms. Most frequently, binary logarithms are used, and the magnitude of entropy is expressed in binary units--bits.

Another situation which is particularly important is that the methods of the theory of information can be used to compute the entropy not only for one indicator, but rather to compute the combined entropy for a whole group of indicators characterizing the state of one or several systems of the organism.

It is shown in the theory of information that the entropy of a combined system of random quantities is equal to the entropy of one of its component parts plus the apparent entropy of the second part with respect to the first

$$H(X, Y) = H(X) + H(X / Y).$$

Let us examine the concept of apparent entropy by using a physiological example. As we have already stated, table 1 presents the data on the pulse rate and the respiration rate recorded in the same person under relatively constant external conditions.

The method of computing the entropy of pulse rate is presented above. Now we shall compute the total apparent entropy of the respiration rate. /212 This means that we shall compute the entropy of the respiration rate under condition that the pulse rate has already assumed definite values, arranged as shown in table 4.

We can see from this table that corresponding to a definite pulse rate (for example, to the interval 71-75) there are several groups of respiration rates (8-10, 11-13, 14-16, 17-19), in which the number of cases is, respectively, equal to 2.6; 7.1, which corresponds to probabilities 0.04, 0.12, 0.14, 0.02. Under these conditions the apparent probabilities RR_j (RR_j are groups of intervals which break down the respiration rate values when the pulse rate is in the third interval PR_3) are equal, respectively, to

$$P(RR_1/PR_3) = \frac{0.04}{0.32} = 0.125\ P(RR_2/PR_3) = \frac{0.12}{0.32} = 0.375, \quad P(RR_3/PR_3) = \frac{0.14}{0.32} = 0.438,$$

$$P(RR_4/PR_3) = \frac{0.02}{0.32} = 0.062$$

[according to equation $P(Y_i/X_i) = P_{ij}/P_i$].

From this the apparent entropy of RR, when the pulse rate varies in the interval 71-75 (PR_3), is equal to

$$H(RR/PR_3) = -\sum_{j=1}^{4} P(RR_j/PR_3) \log P(RR_j/PR_3) =$$

$$\sum_{j=1}^{4} \eta [P(RR_j/PR_3)] = \eta(0.1250) + \eta(0.3750) + \eta(0.4380) + \eta(0.0620) = 1.6760,$$

where $\eta(P_к) = -P_к \log P_к$.

In the general case the apparent entropy Y for indicators X and Y, when X has assumed value X_i, is equal to

$$H(Y/X_i) = \sum_{j=1}^{m} \eta [P(Y_j/X_i)].$$

To determine the total or average entropy of the random quantity, assuming that the random quantity X may assume various values, it is necessary to multiply each apparent entropy $H(Y/X_i)$ by the probability of the respective value of P_i and add all the products. Then $H(Y/X) = \sum_{i=1}^{n} PiH(Y/X_i)$ or after

TABLE 4. COMBINED DISTRIBUTION OF CARDIAC CONTRACTION RATE (PR) AND RESPIRATION RATE (RR) OBTAINED FOR SUBJECT K DURING REPEATED RECORDINGS.

Respiration rate (RR_j)	Pulse rate (PR_i)				No. of cases	Probability
	61-65* (PR_1)	66-70 (PR_2)	71-75 (PR_3)	76-80 (PR_4)		
8-10 (RR_1)	2(0.04)	2(0.04)	2(0.04)	0(0)	6	0.12
11-13 (RR_2)	2(0.04)	5(0.10)	6(0.12)	1(0.02)	14	0.28
14-16 (RR_3)	2(0.04)	11(0.22)	7(0.14)	3(0.06)	23	0.46
17-19 (RR_4)	0(0)	6(0.12)	1(0.02)	0(0)	7	0.14
No. of cases	6	24	16	4	50	0.14
Probability, P_i	0.12	0.48	0.32	0.08	1.00	-

Note. At points where columns and lines intersect we show the number of cases when the system of random quantities (PR, RR) is in the state (PR_i, RR_j), while we indicate in brackets the corresponding probabilities (P_{ij}). *(75 in original text.)

transformation $H(Y/X) = \sum_{i=1}^{n} \sum_{j=1}^{m} P_i \eta[P(Y_j/X_i)]$.

Let us compute the total apparent entropy of the respiration rate. To do this we prepare a table for the apparent probabilities of respiration rate (table 5). Substituting the values of the apparent probabilities into the equation for the apparent entropy we obtain

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$$\begin{aligned} H(RR/PR) &= 0.12\,[\eta(0.3333) + \eta(0.3333) + \eta(0.3333)] + \\ &+ 0.48\,[\eta(0.0843) + \eta(0.2083) + \eta(0.4583) + \eta(0.25)] + \\ &+ 0.32\,[\eta(0.1250) + \eta(0.3750) + \eta(0.4375) + \eta(0.062)] + \\ &+ 0.08\,[\eta(0.25) + \eta(0.75)] = 1.6494. \end{aligned}$$

In the same way, by computing the apparent probabilities of the pulse rate (table 6), we compute the apparent entropy of the pulse rate H(PR/RR) = 1.5485.

The combined entropy for the pulse rate and the respiration rate is equal to H(PR, RR) = H(PR)+ H(RR/PR) = 1.6929 + 1.6494 = 3.3418.

The theorem for the addition of entropy can be extended to two or more numbers of random quantities.

As an example we present data shown in table 7.

TABLE 5. APPARENT PROBABILITIES OF RESPIRATION RATE $\left[P\left(RR_j/PR_i\right)=\frac{P_{ij}}{P_i}\right]$ WHEN PULSE RATE ASSUMES VALUES FROM PR_1 TO PR_4.

Respiration rate (RR_j)	Pulse rate (PR_1)			
	61-65 (PR_1)	66-70 (PR_2)	71-75 (PR_3)	76-78 (PR_4)
8-10 (RR_1)	0.3333	1.0833	0.1250	0
11-13 (RR_2)	0.3333	0.2083	0.3750	0.2200
14-16 (RR_3)	0.3333	0.4583	0.4375	0.7500
17-19 (RR_4)	0	0.2500	0.0625	0

TABLE 6. APPARENT PROBABILITIES OF PULSE RATE $\left[P\ PR_i/RR_j=\frac{Pij}{r_j}\right]$ WHEN RESPIRATION RATE TAKES ON VALUES FROM RR_1 TO RR_4.

Respiration rate (RR_j)	Pulse rate (PR_i)			
	61-65 (PR_1)	66-70 (PR_2)	71-75 (PR_3)	76-80 (PR_4)
8-10 (RR_1)	0.333	0.333	0.333	0
11-13 (RR_2)	0.143	0.357	0.429	0.071
14-16 (RR_3)	0.087	0.478	0.304	0.131
17-19 (RR_4)	0	0.857	0.143	0

From the data presented in the table we see that the combined entropy /214 H(rr, PQ, QT) during the entire flight period has a tendency to become smaller, which apparently points to an improvement in the control of cardiac activity produced by adaptation to conditions of weightlessness.

The theory of information also has a criterion which makes it possible to study the interrelation between random quantities. This criterion is the total mutual information by which the amount of information concerning the random quantity X contained in the other random quantity Y can be determined (in other words, it is possible to determine the total elements for the realization of the random sequence).

Proceeding from the general proposition that the quantity of information is measured by a decrease in entropy when data are received concerning the test system, the criterion for the total mutual information $J_{x \leftrightarrow y}$ is determined as the decrease in the entropy of the random quantity X (random sequence) produced by the receipt of data concerning another random quantity.

TABLE 7. DYNAMICS OF COMBINED ENTROPY H(rr, PQ, QT) OF ELECTROCARDIOGRAM TIME INDICATORS FOR A. G. NIKOLAYEV DURING ORBITAL FLIGHT (ACCORDING TO 24-HOUR FLIGHT PERIODS).

24-hour flight periods	H (rr, PQ, TQ)	F_{exp}	$F_{0.05}$
1	10.10		
2	9.95	1.23	1.34
3	9.79	1.54	1.29
4	9.12	3.89	1.29

Note. F_{exp} is the value of the Fisher criterion obtained from experimental data for cases when the combined entropy is taken with respect to the first 24-hour flight periods. $F_{0.05}$ is the tabulated value of the same criterion when the level of significance is 0.05.

$J_{x \leftrightarrow y} = H(Y) - H(Y/X)$, whereas we have already pointed out that $J_{x \leftrightarrow y}$ is the total mutual information which X contains in itself about Y or Y contains about X.

It is shown in the theory of information that when the test quantities are independent, the total mutual information is equal to 0. To compute the total mutual information directly we use equation (ref. 3)

$$J_{x \leftrightarrow y} = \sum_{i=1}^{n} \sum_{j=1}^{m} P_{ij} \log \frac{P_{ij}}{P_i r_j}.$$

However, if quantities H(Y) and H(Y/X) are known, the computation /215 $J_{x \leftrightarrow y}$ is simply reduced to the subtraction of the first quantity from the second. Thus, in our example H(PR) = 1.6929, H(PR/RR) = 1.5485, and it follows that $J_{PR \leftrightarrow RR}$ = H(PR) - H(PR/RR) = 1.6929 - 1.5485 = 0.1444.

If we proceed from the hypothesis of the normal distribution of random test quantities (random sequences), the total mutual information may be represented as a function of the correlation coefficient

$$I_{x \leftrightarrow y} = \log \frac{2}{\sqrt{1 - r^2}} = -\frac{1}{2} \log (1 - r^2).$$

The magnitude of the criterion for the total mutual information as well as the correlation coefficient can be used in the analysis of physiological data obtained during spaceflights to evaluate quantitatively the compatibility in the activity of the test systems of the organism. Unlike the correlation coefficient, the interrelation between the systems may be evaluated quantitatively in terms of binary units rather than abstract units, which is more illustrative. However, the criterion for the total mutual information does not make it possible for us to determine whether the investigated mutual coupling is positive or negative, and in this respect it is not as good as the correlation coefficient.

In addition to the amount of information carried by a sequence of one physiological indicator concerning another, in a series of cases it is of interest to consider the more general question of the amount of information contained in two random sequences X, Y about a third sequence Z. On the one hand, this makes it possible to obtain information on the interrelation of three indicators and, on the other hand, by knowing the criterion for the total mutual information $J_{X\leftrightarrow Y}$, and also $J_{Z\leftrightarrow(X,Y)}$, we can simply compute the entropy for the system of indicators X, Y, Z.

The calculation of the total mutual information, contained in the duration of the pulse interval RR about the interval QT($J_{QT\leftrightarrow RR}$) of A. G. Nikolayev's electrocardiogram, has shown that during the period of the orbital flight it increased as a function of time span in the state of weightlessness and had the following values over 24-hour flight periods, respectively: 0.08; 0.10; 0.30; 0.25 binary units. During the preflight period it was 0.03 binary units. This nature of the dynamics of mutual coupling between the intervals rr and QT during the flight period apparently points to an improvement in the coordination of the cardiac cycle. However, it is not excluded that the reaction of this type results from an improvement in conditions for blood circulation because of absence of the hydrostatic pressure coefficient.

We have already stated that H(X, Y) = H(X) + H(Y/X); however, it follows from the equation $J_{X\leftrightarrow Y} = H(Y) - H(Y/X)$ that $H(Y/X) = H(Y) - J_{X\leftrightarrow Y}$. /216

From this it follows that $H(X, Y) = H(X) + H(Y) - J_{X\leftrightarrow Y}$ or

$$H(X, Y) = H(X) + H(Y) + {}^1/_2 \log (1 - r^2_{X-Y}).$$

In turn, the entropy of the combined system of random quantities X, Y, Z may be represented in the form

$$(H/X, Y, Z) = H(X, Y) + H(Z) - J_{Z\leftrightarrow(x,y)} = H(X, Y) + H(Z) + 1/2\log[1 - r^2_{Z(X-Y)}].$$

$$H(X, Y, Z) = H(X) + H(Y) + H(Z) + {}^1/_2 \log (1 - r^2_{X-Y}) + \frac{1}{2}\log [1 - r^2_{Z(X-Y)}].$$

The last equation makes it possible to express the combined entropy for three random quantities (sequences) in terms of the entropy of each quantity by itself and in terms of the correlation coefficients $r_{X - Y}$ and $r_{Z(X - Y)}$.

We should point out that as in the case of multiple correlation this equation is valid only when we have a normal distribution of the quantities.

In the quantitative evaluation of physiological data we encountered the important problem of verifying the zero hypothesis and the equality of entropies and criteria for the total mutual information.

This question, if we accept the hypotheses on the normal distribution of the investigated random sequences, is reduced to the verification of the zero hypothesis concerning the equivalence of dispersion (Fisher's criterion) and the correlation coefficients (the Z-transform of Fisher). However, the Fisher criterion may be expressed directly in terms of entropy

$$H = \log \frac{\sqrt{2\pi e \sigma^2}}{\Delta X}.$$

Solving the equation with respect to σ^2 we have $\sigma^2 = 2^{2H} \cdot \frac{\Delta x}{2\pi l}$. It follows from this that $F = \frac{2^{2H_1}}{2^{2H_2}} = 2^{2(H_1 - H_2)}$.

In the same way it is possible to check the zero hypothesis and the equivalence of combined entropies of the indicators over the different regions.

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THE POSSIBILITY OF UTILIZING ELECTRONIC LOGIC CIRCUITS FOR AUTOMATIC MEDICAL MONITORING

V. Ya. Kostikova, R. M. Bayevskiy, A. P. Kalinovskiy
and B. A. Soshin

One of the basic problems of space engineering and space biology is /217 to provide for the safe flight of man into space. Among the different investigations concerned with this problem, an important place is occupied by the problem of developing medical control over the passengers in a spacecraft. The reception of information required by the physician to make a "diagnosis" is associated with the utilization of a large number of radio channels, rapid decoding of all data and their generalization. The evaluation of information and the adoption of solutions must be done so quickly that it is outside the capability of the human nervous system. Therefore, in order to provide for a truly effective and reliable medical control it is necessary to use automation facilities whose problem is to determine the quantitative values of biomedical indicators transmitted over each channel, to compare them by means of a given logical circuit and to develop a code showing the state of the cosmonaut (ref. 1).

The increase in the duration and range of spaceflights will produce a sharp decrease in the capacity of radio channels and transmission time. It is expected that during lunar or interplanetary flight the carrying capacity of the radio telemetry system will be insufficient to transmit biomedical indicators in their conventional form. Therefore, the development of automatic devices, carried aboard the spacecraft to generalize data on the state of the cosmonaut, is part of the long-range and urgent problems (ref. 2).

At the present time there are two types of automatic devices for processing information: systems operating with a fixed program (electronic logic /218 circuits) and systems whose programming is flexible and can be varied during the process of operation (computers). Both of these devices can be of the analog or digital type. In the present work we shall consider the possibility of applying electronic logic circuits for the automation of medical control during spaceflight.

The application of systems carried aboard the spacecraft and operating with a fixed preassigned program is not very suitable for cosmic flights of long duration and distance, where it is necessary to rearrange the programs for the automatic processing of data in accordance with the changing flight conditions and the state of passengers. However, for short flights over established routes such systems are entirely applicable. The advantage of electronic logic circuits is their relative simplicity, low weight and small size.

We shall consider the principles of developing an automatic device to be carried aboard the spacecraft, designed to solve a logic diagnostics problem whose algorithm is assigned in a matrix form. To provide for effective automatic medical control it is necessary to give serious attention to the selection of medical parameters introduced into the data handling system. To provide for reliability of medical control during flight the sensors and electrodes must be permanently attached to the cosmonaut and must introduce high quality information into the automatic data handling system at any time during the flight.

However, not all physiological parameters which are desirable for control purposes can be recorded in a stable manner under conditions of spaceflight of sufficiently long duration. Thus, for example, the experience of recording the electroencephalogram during the flights of A. G. Nikolayev and P. R. Popovich has shown that, in spite of the high quality of recordings obtained during the periods when the cosmonauts were in a state of relative rest, certain sections of the curve had to be excluded from the analysis because they were distorted by motion and myogenic biocurrents.

It is clear that the separation of noise is possible only by means of special, rather bulky devices or by a specially programmed computer. Therefore, the introduction of the electroencephalogram into electronic logic devices, in spite of its importance, is not readily achieved because of the high complexity of the input device. The reliability of sensors and the simplicity of the input device are important conditions for the development of electronic logic systems carried aboard the spacecraft. A rather important criterion is the selection of biomedical indicators which would be sufficient to produce a "diagnosis" (to detect dangerous deviations in the state of the cosmonaut). Thus, in order to diagnose the loss of consciousness under conditions of spaceflight, it is necessary to know at least the pulse rate (PR), the respiration rate (RR), /219 body temperature (BT), state of consciousness (SC), the level of motor activity (MA). Additional data can be obtained by measuring the electrical skin resistance (ESR) and also the air temperature (AT), as well as oxygen and CO_2 content

(the last three parameters are important for differential diagnostics).

All these measurements can be carried out by means of rather simple methods, and only two types of circuits are necessary to feed them into an electronic logic device: one circuit is required for the conversion of digital indicators and the second for the conversion of indicators which vary continuously with time. In the general form the algorithm for analyzing each of these indicators includes only three operations:

(1) the measurement of the indicator during a given period of time (for example, the measurement of the number of pulses during a period of 10 sec or the average voltage level during the same period of time);

(2) the comparison of values obtained with the preassigned limits for the norm and the determination of the instantaneous state of each indicator (for example, in the form of symbols "N", "+" and "-", depending on whether an indicator is within a given range or whether it has increased or decreased); and

(3) the correlation of the symbols for different parameters according to a fixed logic scheme and the identification of the code-"diagnosis".

Let us consider specific engineering approaches for the realization of these operations. The construction of reliable measurement circuits is associated first of all with the reliability of their sensors. The sensors of a measurement system, which provide for the introduction of the necessary information into electronic logic devices, must be placed on the body of the cosmonaut and must operate in a stable fashion under all conditions, even when the cosmonaut is moving (ref. 3). To measure a group of parameters to diagnose the loss of consciousness, special recording methods and special methods of mounting sensors were developed. The pulse rate and the respiration rate, as well as the electric skin resistance, were measured by using techniques developed to record electrocardiograms, pneumograms and cutaneogalvanic reactions aboard spacecraft of the "Vostok" series (ref. 4). The skin temperature was measured by means of several thermistors placed on various regions of the thorax. Measurement of the motor activity was carried out by counting the number of pulses developed by a critically damped seismic sensor strapped to the chest. The state of consciousness was determined by the presence or absence of a reply reaction to a conditioned signal.[1]

Two types of circuits were used to compare current values with assigned values. A digital system was used to analyze information flowing through the channels for measuring pulse rate, respiration rate and motor activity. The block diagram of this system is shown in figure 1. The basic unit of this system is a binary counter, controlled by a one shot multivibrator. This makes the operation of the counter independent of pulse amplitude. The circuit has a 10 sec timing generator which controls the binary counter--every 10 sec all digits of the counter are cleared to the 0 position and the counting starts over again. Thus, at the end of each 10 sec period the counter displays a binary number which corresponds to the number of pulses accumulated just before the digits are cleared. /220

After the counter is cleared the new 10 sec cycle of measurement starts. The number of digits in the binary counter is determined by the possible range of the measured parameter frequency. Thus, if we assume that the largest number of pulses which can be measured during a 10 sec period is equal to 50, which corresponds to 300 cardiac contractions per minute, the binary counter must have not less than six digits. /221

The frequency of pulses is analyzed by a system of code registers. The binary numbers corresponding to the extreme values of the measured parameter are set in the register by means of a keyboard. Thus, if the entire range of possible variations is divided into three parts (below normal, normal, above normal), it is necessary to have two code registers. A number corresponding to the lower boundary of the norm is set on one of them, and the number corresponding to the upper boundary of the norm is set on the other. The numbers in the

[1]The method and sensors for measuring the skin temperature, the motor activity and the response reactions were developed by R. M. Bayevskiy, V. V. Bogdanov and L. A. Kazar'yan.

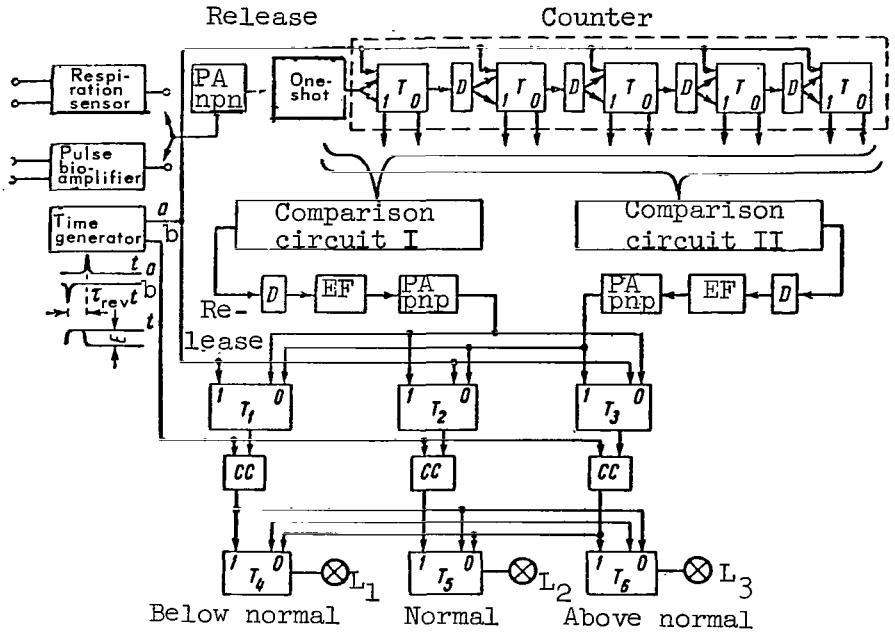

Figure 1. Block diagram of system for analyzing discrete indicators. PA, pulse amplifier; T_{0-6}, flip-flops; D, differentiating network; EF, emitter follower; CC, coincidence circuit; $L_{1, 2, 3}$, electric lamps, 6V.

code registers are compared with the numbers in the binary counter by means of comparison circuits. Each comparison circuit handles its code register (fig. 2). The outputs of the comparison circuits are fed to output flip-flops. Depending on the frequency of the arriving pulses, a signal appears at one of the output flip-flops at the end of the 10 sec period.

The diagrams shown in figures 3 and 4 are used to analyze the information transmitted over the channels for measuring body temperature, electric resistance and hygienic parameters.

The input signal has the form of a slowly varying voltage. The measurement of the input signal level is cyclic, and the period of the cycle is determined by the frequency of the triggering pulse generator, which consists of a free-running multivibrator. As before, the period of each cycle is 10 sec.

Two square-wave pulses are picked up from the outputs of the multivibrator. One of these is used to fire the reference voltage multivibrator, while the other is used to reset the flip-flops (Tr_1, Tr_2) of the output /222

device. The signals taken from the output device reflect the level of the measured voltage, i.e., they show whether it is within its normal limits,

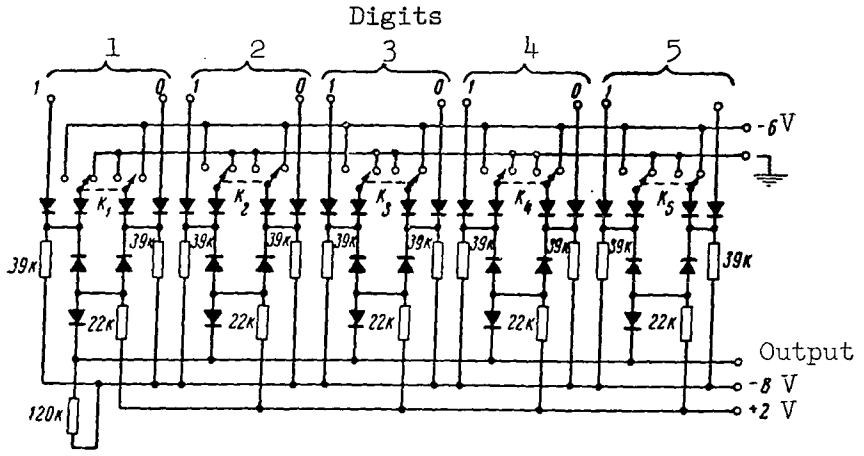

Figure 2. Comparison circuit.

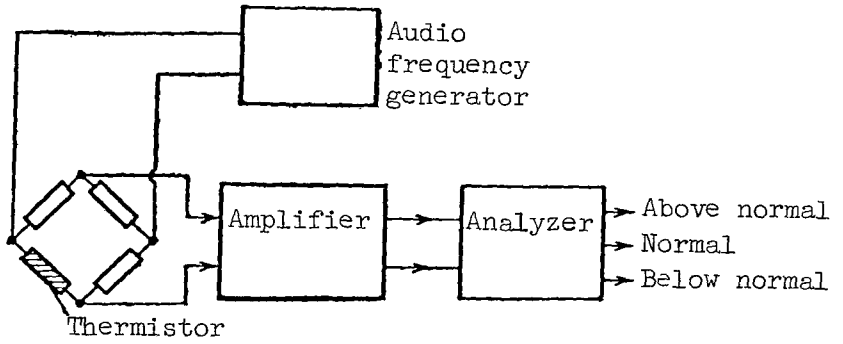

Figure 3. Block diagram of system for analyzing continuously flowing indicators.

below these limits or above. The respective outputs are designated by "N," "-," "+." When the triggering pulse appears at the reference voltage multivibrator, a positive step voltage appears at its output, and this voltage is fed to the comparison circuit. The measured voltage is also fed to the comparison scheme through terminals A and B. As a result of the comparison operation, pulses are fed to the flip-flops Tr_1 and Tr_2 of the output circuit, and the amplitude of these pulses is determined by the difference in the level between the measured voltage and the voltage taken from the reference flip-flop Tr_3. Depending on the magnitude of the triggering pulse amplitude, the flip-flops Tr_1 and Tr_2 may or may not switch. These flip-flops in turn control the circuit S_p. The setting of the upper and lower limits for the normal values

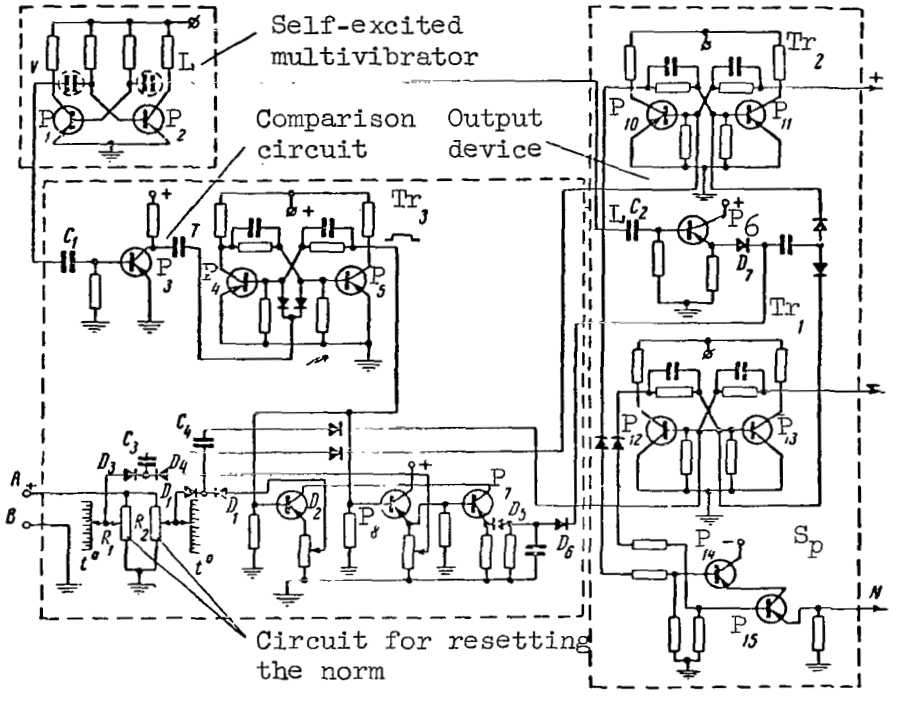

Figure 4. Diagram of analyzer for continuous indicators.

is accomplished by means of potentiometers R_1 and R_2. R_2 is used to set the lower limit, while R_1 is used to set the upper limit.

The use of potentiometers in the comparison circuit has made it possible to simplify the circuit as a whole.

When the magnitude of the measured voltage is below the lower limit of the norm, a negative voltage appears at the output designated by the symbol "-." The outputs "+" and "N" are at zero potential. When the measured voltage reaches normal values, a negative voltage appears at the output "N", while the outputs "+" and "-" will be at zero voltage, etc.

Thus, at the output of each measurement channel we can obtain the symbols "N," "-" or "+" given by the states of the output flip-flops. In the channel for recording the response reaction provision is made for two possible output signals "N" and "$\bar{N}$" (normal or not normal). The total number of signal /223

combinations at the output of all 10 channels is equal to $3^3 \times 2 = 13{,}122$. The problem consists of selecting a definite number of combinations which would correspond to the probable danger state of the cosmonaut. This is a typical problem in medical programming.

However, although diagnostic programs for clinical application are being developed at the present time with considerable success, this problem is considerably more difficult under conditions of spaceflight. The fact is that the influence of spaceflight factors on the human organism has not been studied in sufficient detail, and the preparation of tables for syndromes-diagnoses is carried out primarily on the basis of laboratory data and theoretical analysis.

Since the purpose of the present article is to consider the technical rather than the medical aspects of automatic medical control, we shall limit ourselves to an example where the program is presented in matrix form (table). The codes designated by the digits 1, 2, 3, show that a loss of consciousness is taking /224 place as evidenced by the coincidence in the deviation of the ESK, OR and D. The fourth code (0) corresponds to the normal state. Certain properties in the reaction of pulse and respiration and the variation in hygienic parameters make it possible to differentiate the loss of consciousness from the causes which produce it. This substantially increases the effectiveness of the assumed solution. Figure 5 shows the logic circuit--a diode matrix, which is programmed in accordance with the table. The general block diagram of the device for the automatic medical control is shown in figure 6.

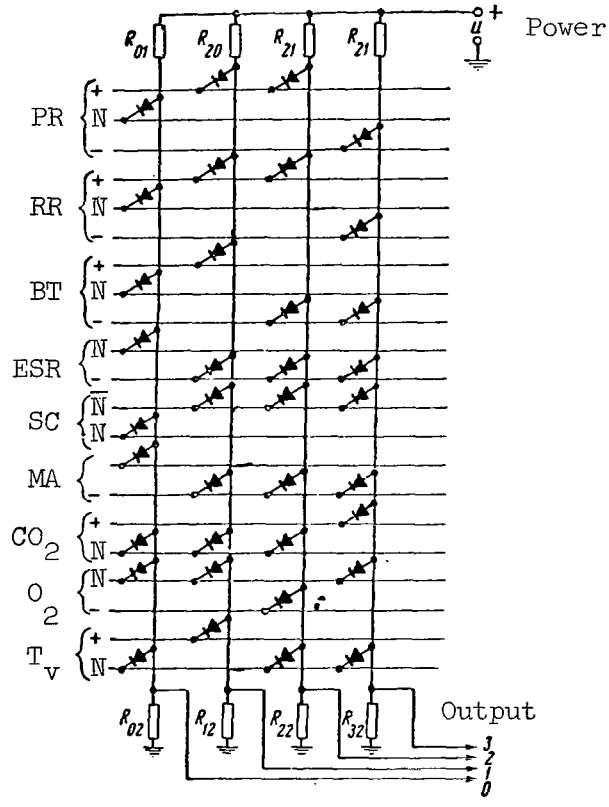

Figure 5. Logic circuit consisting of diode matrix.

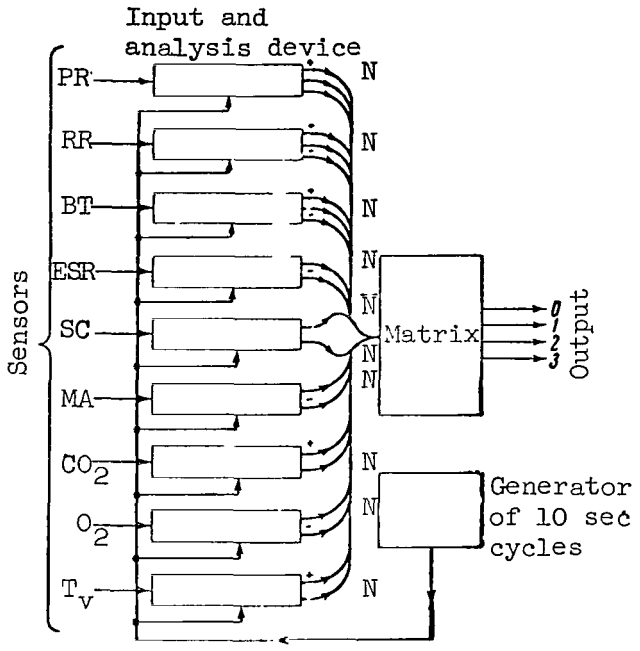

Figure 6. Block diagram of electronic logic device for automatic medical control.

Devices based on electronic logic circuits have the following features:

(1) they permit continuous monitoring of a rather large number of indicators with a presentation of any of their assigned values;

(2) they provide for generation of a signal when any desired combinations of the deviations appear;

(3) they provide for a rather simple engineering solution of the problems associated with automatic medical control.

The electronic logic circuits may be used in cases when there is prior knowledge of the relationship between the factors influencing a man and the functions of the organism (in other words, all of the problems of automatic "diagnosis" which can be treated by the method of linear programming, can be solved expediently by means of electronic logic circuits).

As we know, each spaceflight is preceded by hundreds of laboratory /226 experiments. The results of these experiments can be used for programming electronic logic circuits aboard the spacecraft. It is also clear that the utilization of such systems in laboratory experiments is an important step toward the introduction of automatic medical control in the practice of space biology.

OPERATIONAL PROGRAM OF ELECTRONIC LOGIC CIRCUITS

Code	Parameters								
	PR	RR	BT	ESR	SC	MA	CO_2	O_2	T_v
1	+	+	+	-	$\overline{N}$	-	N	N	+
2	+	+	-	-	$\overline{N}$	-	N	-	N
3	-	-	-	-	$\overline{N}$	-	+	N	N
0	N	N	N	N	N	N	N	N	N

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PART II. RESULTS OF EXPERIMENTS CONDUCTED ON ORBITAL SPACECRAFT

SOME PHYSIOLOGICAL DATA FOR EVALUATING THE CONDITION AND WORK CAPACITY OF COSMONAUTS IN ORBITAL FLIGHT

A. D. Voskresenskiy, O. G. Gazenko, G. V. Izosimov, V. I. Kopanev, D. G. Maksimov and V. I. Yazdovskiy

Each new flight of man into space is a unique scientific experiment /227
conducted in pursuit of manifold investigative goals. Among the basic biomedical tasks are accumulation and analysis of data which permit evaluation of the condition and work capacity of the cosmonaut in the course of his flight. Information from several sources is used for this purpose.

An overall grasp of the effects of various flight factors on human work capacity may be obtained on the basis of returns from the cosmonaut, checks on fulfillment of in-flight assignments, television and radio-conversation data, recordings from onboard tape recorders, etc.

Certain special methods also give valuable material: controlled stimuli of the vestibular apparatus, tests on motor coordination and psychological tests (refs. 8, 9 and 12).

However, complete and objective analysis of human behavioral dynamics during prolonged spaceflight can be achieved only by the interworking of a complex of methods used in general physiology, which permits assessment of the state of the higher nervous activity and functional shifts in the work of separate sensors (refs. 1, 2 and 6). After the flight of G. S. Titov, in which the cosmonaut manifested several symptoms of vestibular distress, it became evident that further studies were essential.

Of greatest importance in monitoring the functional state of the /228
higher sectors of the central nervous system is registration of bioelectric activity in the cerebral cortex. EEG telemetry during spaceflight was first accomplished for the Vostok-3 and Vostok-4 spacecrafts of A. G. Nikolayev and P. R. Popovich. Silver electrodes were used as pickups, located on the forehead and occiput. The same method of recording EEGs was employed in the flights of V. F. Bykovskiy and V. V. Tereshkova.

Besides this, galvanocutaneous reactions of the cosmonauts were recorded on the Vostok-3 and Vostok-6 flights. These galvanocutaneous reactions (GCR) were studied by the Ferrer exosomatic method. Electrodes were pasted onto the lower extremities. The GCR recording circuit proposed by I. T. Akulinichev ensured high sensitivity to changes of short duration, as well as great amplification of slow daily oscillations in resistance.

Slow changes in electrical resistance of the skin were registered during the Nikolayev-Popovich flight from the amount of circuit output voltage as an inverse function of resistance. Relatively fast changes in resistance were recorded in Bykovskiy and Tereshkova under the influence of external stimuli. The number of reactions and, in some cases, their duration and amplitude were measured. With the absence of control stimuli it was, of course, impossible to determine the latent period of reaction.

Electrooculographs were taken in the first and second group flights to monitor the functional state of the vestibular apparatus and of the central nervous system as a whole (refs. 3, 11 and 15). Oculomotor activity was recorded, using silver electrodes placed on the skin at the outer palpebral angles, by a method employed previously by several authors (refs. 10, 13 and 14). The difference in corneoretinal potentials, arising during movement of the eyes, depended on the amplitude of the movements and fluctuated between 20 and 280 μV. After preliminary amplification the signal was transmitted to Earth and was recorded on photoblocks.

There is some interest in tracing whether or not there exist any definite tendencies in EEG, GCR and EOG changes and what the connection might be between these changes and both the cosmonaut's personal sensations and the objective indexes of his general condition in flight.

The EEGs of Nikolayev and Popovich during the first day of flight were characterized by high β rhythms and, to a lesser degree, α rhythms. The index of low-frequency oscillations (up to 8/sec) was, on the other hand, relatively low. Consequently, the average values of the indexes in separate portions of the flight fluctuated within narrow limits. The amplitudes /229 of the rhythms remained virtually unaltered.

Sample EEG in-flight records for Bykovskiy are shown in figure 1. We can distinguish low-frequency oscillations of δ rhythm (1-4 per sec) and θ rhythm, (5-7 per sec). The basic α and β rhythms (8-12.5 and 13-25 per sec, respectively) were quite evident. Blinking appeared in the form of slow, high-amplitude, two-phase waves with an oscillatory period of 200 msec.

The magnitude of the α rhythm index on the first to fourth orbits was 45-58 percent, while during the succeeding observations it was 35-40 percent. The index for β rhythm showed significant oscillations, but in most of the recordings from the first and second days of the flight it exceeded 50 percent. The index of low-frequency waves, along with sharp deviations, revealed a tendency to reduction, especially pronounced on the 8th-12th orbits.

In connection with drying of the current-conducting paste and destruction of the contact between the electrodes and the cutaneous surface on the third day of the flight, there was a substantial increase in EEG noise. From the 35th orbit on chiefly fast oscillations were recorded, with a frequency of 17-20/sec. Blinking was not reflected on the EEG. Separate parts of the slow oscillations, as a rule, were associated with the act of inhaling and with movements of the cosmonaut.

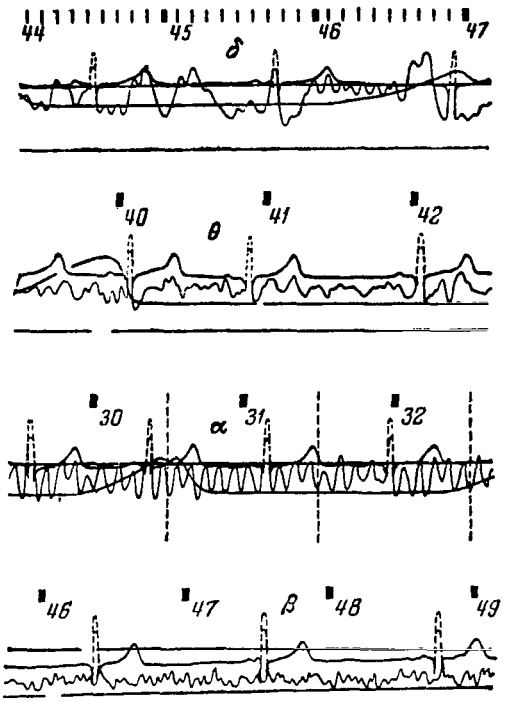

Figure 1. Sample EEG recordings of V. F. Bykovskiy during orbital space-flight. α, β, θ, δ, Typical rhythms of electrical activity of brain.

EEGs of V. V. Tereshkova were more qualitative, which can in part be attributed to her lesser motor activity during the flight. This last is confirmed by data from television observation.

In the one minute before start, rapid frequencies, occurring at in- /230
tervals with low-amplitude α rhythms (2-3 mm, 10/sec), predominated in Tereshkova's EEG. In the active part of the flight, the EEGs were recorded with noise from muscular tension. However, we note in the recordings the presence of a β rhythm with 16-18 oscillations per sec and an amplitude on the order of 2 mm.

On the second and third orbits against a β rhythm background separate slow oscillations (3-4/sec) appear with an amplitude of 3-6 mm and portions of the α rhythm (10-12/sec) with an amplitude of 4-5 mm. On the seventh orbit, prior to going to sleep, Tereshkova several times closed her eyes. This can be judged both from the clear recording of eyelid movement and from the appearance of a marked α rhythm with an amplitude of 8-10 mm. When she opened her eyes, there was a reaction of α rhythm blocking and predominance of rapid oscillations.

On the morning of the second day of the flight (13th orbit) rapid frequencies (16-17/sec) predominated, with an amplitude of 2-3 mm. Later

(17th to 19th orbits) the θ rhythm was strengthened, with an amplitude of 3-4 mm. On the 18th orbit a sharply defined α rhythm with an amplitude of 8-9 mm was recorded when the eyes were closed. On the 20th orbit δ waves appeared against the θ rhythm background, with a frequency of 4 per sec and an amplitude of 3-5 mm. Before sleep the slow oscillations were amplified; waves appeared on the EEG with a frequency of 2-3 per sec and an amplitude of 10-12 mm.

The morning of the third day (29th orbit) was characterized by the presence of low-amplitude θ rhythm oscillations (2-3 mm, 6-7 per sec) on the EEG. On the 32nd orbit, along with periodic holding of the breath, a pronounced α rhythm (8-9 mm, 10/sec) was observed. When the eyes were open, a reaction of α rhythm blocking occurred. After one particularly long holding of the breath after inhalation (10 sec), a δ rhythm (13-14 mm, 3/sec) appeared on the EEG.

On the 33rd and 34th orbits the α rhythm alternated periodically with slow waves (fig. 2). On the 38th orbit, before sleep occurred, groups of slow waves were recorded with a frequency of about 3/sec and an amplitude of 6-7 mm. A β rhythm arose periodically against this background. On the 39th orbit clearly articulated 4-5 mm α rhythms were recorded occasionally. On the last day of the flight, right up to the time of descent, a 1-2 mm β rhythm predominated, with separate sectors of θ rhythm.

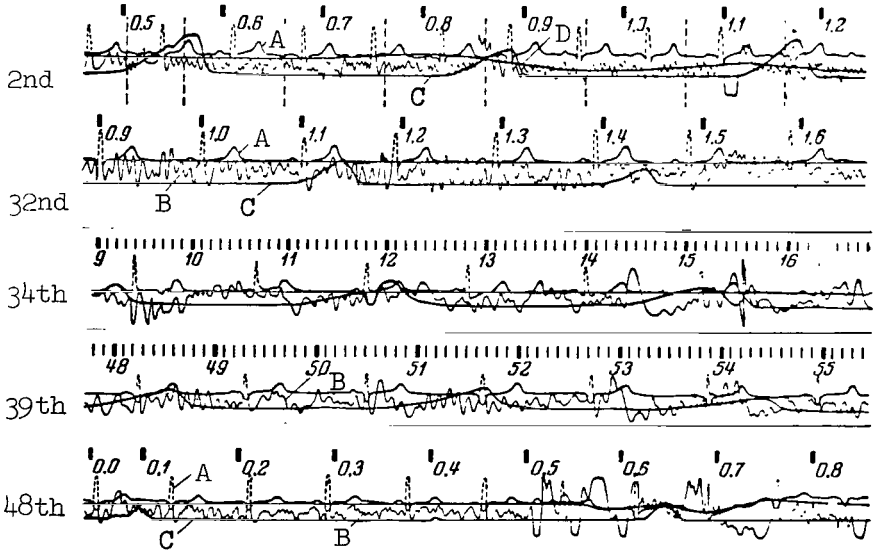

Figure 2. Sample EEG recordings of V. V. Tereshkova during orbital spaceflight. Numbers to left of curves designate flight orbit. 2, Predominance of high-frequency oscillations; 32, α rhythm elevation; 34, slow waves in waking state; 39, slow waves before going to sleep; 48, secondary amplification of high-frequency oscillations at end of flight. Numbers on curves denote time in sec. Simultaneously with EEG (B), recordings were made of EKG (A), changes in perimeter of chest (C) and galvanocutaneous reactions (D).

AVERAGE NUMBER OF GALVANOCUTANEOUS REACTIONS PER MIN FOR V. F. BYKOVSKIY AND V. V. TERESHKOVA DURING SEPARATE PARTS OF THE FLIGHT.*

Cosmonaut	Part of flight						
	5 min before launch	Active part	2nd orbit	18th orbit	38th orbit	45th orbit	48th orbit
V. F. Bykovskiy	6	11	8	5	1	2	6
V. V. Tereshkova	8	12	8	2	2	3	-

*GCR investigations were conducted by A. Ye. Baykov.

As a whole the flight period was characterized by a notable reduction in the β rhythm index and by a less pronounced increase in the θ rhythm index. Only on the last orbit did the specific weight of high-frequency oscillations rise significantly. The α rhythm index during the first two days of flight fluctuated from 25-40, on the third day it rose to 50-70, and during the final period (46th to 48th orbits) it dropped to 30-35 percent.

The value of electrocutaneous resistance gradually dropped over the entire course of Nikolayev's flight. In the case of Popovich a reduction was observed during the first half of the flight, after which the curve rose somewhat. /231 Study of the slow changes in electrocutaneous resistance showed that this index possesses diurnal dynamics, being greater in the morning than the evening. A more pronounced daily rhythm was observed in Popovich. A sharp drop in resistance was noted in the period of greatest emotional stress (start, descent).

The average number of rapid oscillations of the GCR curves per min was computed for Bykovskiy and Tereshkova. These data are presented in the table.

Analysis of the GCR data for Bykovskiy showed a reduction in the average duration of in-flight reaction as compared with the period 5 min before launch. The GCR duration reached the initial level only at the end of the flight (81st orbit). The amplitudes of the positive and negative phases fluctuated in wide ranges, on the average exceeding the level characteristic for the preflight period.

During Tereshkova's 5 min launch alert, and during the first to /232 seventh orbits, the duration of the reaction was 9-13 sec, on the 13th to 23rd orbits it increased, being higher during the first half of the day than before sleep. On the 29th to 39th orbits (second and third day of the flight), the duration of the reaction dropped to 8-10 sec, while before descent it rose to 14-17 sec. The amplitude of the positive phase in the first 1.5 days declined, then attained the initial level and before descent again dropped. The amplitude of the negative phase fluctuated within 3 percent limits.

Analysis of the temperature recordings of the EOGs showed that for all the cosmonauts at the beginning of the flight the number of oculomotor reactions per unit of time rose considerably. The degree of increase exhibited substantial individual differences. Thus, for Nikolayev and Bykovskiy the average number of eye movements per min reached 110-130, while for Popovich and Tereshkova it varied in the range of 40-90.

On the third to seventh orbits a certain reduction in oculomotor activity was noted, with an especially sharp drop on the second and third days of flight. The reduction affected small- and medium-amplitude movements as well as large-amplitude movements.

The first days of the flight were characterized by frequent sharp oscillations in the EOG curve upward and downward from the isoelectric line, which conformed to eye movements to the right and left of the center of vision; then, with reduced activity, individual groups of frequent oscillations of varying amplitude were observed. Later oscillations appeared on the EOG reflecting mostly solitary eye movements. At the end of the flight all cosmonauts manifested secondary heightening of oculomotor activity, which was less pronounced than on the first orbits.

Movement of the eyeballs was basically symmetrical, which indicates the similarity of the functional state of the labyrinthine apparatus of both sides. Appearances of prolonged spontaneous nystagmus affecting gross impair- /233
ments of the functions of the vestibular apparatus were not recorded. Only after increased oculomotor activity, associated with optokinetic stimuli, did we observe adaptive nystagmus of short duration, in the form of 3-7 small-amplitude, saw-tooth oscillations.

V. V. Tereshkova on the 38th and 45th orbits exhibited brief nystagmoid movement of medium amplitude (fig. 3). The resemblance to nystagmus was determined from the length of the effect (10 sec) and oscillatory frequency (2-3/sec), as well as from presence of fast and slow components. However, these movements differed somewhat from the typical picture of nystagmus observed under terrestrial conditions. In particular, a fast and slow component was not sharply articulated in all complexes. At the same time, both in flight and in studies made with Barani chairs, one characteristic feature was isolated: secondary augmentation of amplitude and quickening of the rhythm during conservation of the direction of motion.

The data cited here permit clarification of certain general features /234
in the changes of separate physiological parameters in various phases of the flight.

A distinctive feature of bioelectric activity of the cerebral cortex in the first hours and days of flight was the increase in the index of high-frequency oscillations. According to EEG records submitted for interpretation, the steady or periodic predominance of the β rhythm in Nikolayev, Popovich and Bykovskiy can be observed for at least two days. The index of low-frequency oscillations remained small and did not reveal a tendency to increase. In the

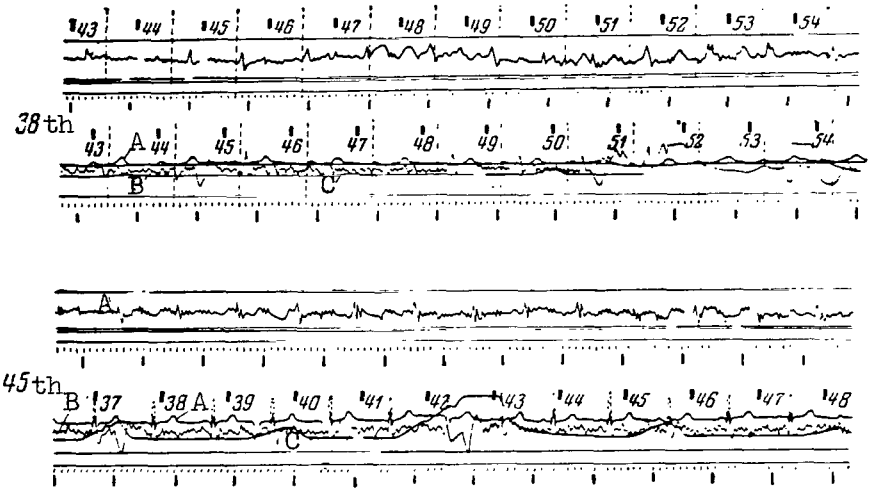

Figure 3. Nystagmoid ocular movements of V. V. Tereshkova during flight. Upper curves denote EOGs recorded on 38th and 45th orbits. Rhythmic nystagmoid ocular motion is evident. Same channel was used to register seismocardiographic complexes (A) consisting of initial high-amplitude and subsequent low-amplitude oscillation. Lower curves represent data recorded at this time for EKG (a), EEG (b), chest perimeter (c) and galvanocutaneous reactions (d).

first day and on the morning of the second, high frequencies predominated also on the EEG records of Tereshkova.

It was characteristic that the initial period of flight was also distinguished by increased oculomotor activity and an augmented number of rapid oscillations of electrocutaneous resistance per unit of time.

These reactions are apparently related to emotional "lift" and a somewhat excited state after successful entry into orbit, acclimatization to which, of course, requires many hours. Such factors as radio conversations with ground communication points and between ships, reception of commands and signals and observation of the surface of the Earth and heavenly bodies could play the role of powerful stimuli supporting the high level of psychoemotional reactions.

On the other hand, the action of an entire complex of stimuli, extraordinary both in strength and character, in conjunction with a plethora of in-flight duties, can obviously lead after a certain amount of time to symptoms of fatigue and slowing down of the central nervous system.

In experiments with isolation lasting for days, under condition of restricted space, the development of early forms of fatigue appears in the test subjects, indicated by a certain shift in the EEG frequencies to slower rhythms (ref. 4). In this repspect it is interesting to note the appearance on Tereshkova's EEGs of slow-activity waves in the daytime, with an increase in their specific

weight from the second to the third day of flight. Although Tereshkova remained alert and manifested no diminution of work capability, the EEG changes evidently can be regarded as an objective symptom of the process of adjustment of the functions of the central nervous system, which during more prolonged flight might be accompanied by pronounced feelings of fatigue. However, in evaluating the EEG data we must keep in mind that synchronization of the biopotentials of the brain, which generally are considered a sign of strengthening the inhibitory processes, can also reflect a shift toward excitation (refs. 5 and 7). /235

The drop in oculomotor activity below the initial level is probably another symptom with analogous admonitory significance. Secondary strengthening and quickening of eye movements in the last orbits of flight can be explained by emotions associated with the expectation of descent. It is remarkable that in this period Tereshkova's EEG graph changed. The specific weight of slow waves declined and, as at the beginning of the flight, the β rhythm again predominated.

It is worthy of note that the sharp limitation of eye movements, nystagmoid phenomena and pronounced amplification of slow waves appeared on Tereshkova's EEGs almost simultaneously on the third day of flight. Although the cosmonaut did not experience subjective symptoms of motion sickness, we consider the cotemporality of these factors in some measure related to a specific effect of weightlessness on the vestibular apparatus.

This effect, apparently, did not reach an extent capable of evoking noticeable autonomic damage or reduction in work capability. However, the unusual condition of the functioning of the vestibular apparatus in circumstances of weightlessness are confirmed by subjective impressions of the cosmonaut. Thus, P. R. Popovich indicated that he did not sense any vestibular and autonomic disorders, but during sharp declination of the torso forward he experienced each time a sensation reminiscent of sensory reactions on the revolving chair (ref. 9).

Thus, the EEG, GCR and EOG data substantially supplement our concepts of the influence of factors of prolonged spaceflight on the overall condition of the cosmonaut. The dynamics of these parameters can reflect a process of psychoemotional adaptation of man to flight conditions. EEG changes and the sharp reduction in oculomotor activity can apparently serve as objective admonitory symptoms of approaching fatigue. The EOG data also permit a sounder judgment regarding the effects of weightlessness on the function of the vestibular apparatus. It is important to emphasize that the EEG, GCR and EOG changes observed during spaceflight were not accompanied by subjective feelings of impending fatigue, by vestibular symptoms or by a noticeable decrease in work capability.

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SOME RESULTS OF BIOMEDICAL INVESTIGATIONS CONDUCTED DURING THE TRAINING PERIOD AND FLIGHTS OF COSMONAUTS V. F. BYKOVSKIY AND V. V. TERESHKOVA

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The era of spaceflights that was auspiciously inaugurated on /237
April 12, 1961, by Yu. A. Gagarin has developed rapidly. Indeed, within 23 days of the flight of Vostok, the American Alan Shepard completed the first suborbital flight in the United States in the Mercury capsule (MR-3). By June 1963 a substantial mass of information had accumulated on the effect of spaceflight factors lasting up to 4 days on male pilots. Four orbital flights had been completed by this time in the Soviet Union, involving more than 192 hours of weightlessness, as well as 2 suborbital and 4 orbital flights in the United States with a total duration of about 54 hours.

Analysis of the data led to preparations for longer flights by men and to initial planning for the training of women. As part of the program for studying and mastering space, the next Soviet flight with cosmonauts V. F. Bykovskiy (Vostok 5) and V. V. Tereshkova (Vostok 6) took place on June 14-19, 1963. The principal medical tasks in connection with these flights included: (1) further study of the effect of prolonged spaceflight factors on the human body; (2) study of the psychophysiological capabilities and efficiency of human beings when exposed to prolonged weightlessness combined with other factors; (3) investigation of the reactions of the female organism to spaceflight conditions; (4) further study of the daily physiological rhythms of man during space- /238
flight; (5) study of the effectiveness of the methods of selecting and training cosmonauts; (6) analysis of the quality of biomedical supervision of the cosmonauts and of the microclimate in the spacecraft cabin; (7) study of the performance of the life-support systems and safety devices in the spacecraft.

In accordance with the foregoing, a program was worked out whereby the cosmonauts had to control the spacecraft manually; check the functioning of the onboard apparatus; conduct observations of Earth, Moon, Sun and other heavenly bodies through an illuminator and by means of optical systems; take motion pictures; perform biological and physical experiments; maintain radio contact with Earth and with each other; carry out medical self-observation; regularly take psychological, physiological and vestibular tests; accurately record the results of their observations in the logbook and on magnetic tape. In addition, during certain orbits they had to detach themselves from the chair and "float" freely about the cabin while performing some operations.

Training of the Cosmonauts

The scope and nature of the cosmonaut training program was determined largely by the objectives of the forthcoming joint flight.

A good deal of experience had been gathered in training men for prolonged spaceflight, but difficulties arose in connection with women, due to their anatomical and physiological features.

Physicians had difficulty in selecting cosmonauts from among the candidates, because the concept of a "healthy" person does not tell everything about the characteristics which indicate tolerance of external (especially space) factors and the possibility of adapting to them. Yet it was necessary to find those most capable of resisting stress influences. Besides observing the reactions to numerous stimuli, attention was focused on the compensatory capabilities of the organism with respect to intensifying and repeated mental and physiological loads.

Thus, the physicians discovered the peculiar internal resources of the female organism which are mobilized as environmental conditions deteriorate /239 or when great functional demands are made on the organism.

Special methods were used to investigate the nature of the interaction of the sensors responsible for the well-coordinated functioning of the "man-machine" system in controlling the spacecraft.

What is particularly important here is the rapidity with which stimuli are perceived and the appropriate reactions elicited. The interaction of the sense organs may be reflected in disinhibition or, contrariwise, inhibition of each sensor separately. This makes it necessary to introduce corrections in the existing methods of investigating the sensors. However, the phenomena observed suggest that efforts should be made to inhibit injurious reactions in spaceflight.

The next stage in the selection system was to study each candidate psychologically in order to discover emotionally stable individuals with swift general reactions, ability to concentrate, good memory and ability to make deliberate coordinated movements in short periods of time.

The methods of investigation, generally speaking, made it possible to select candidates with all of these desirable qualities for cosmonaut training.

The purpose of the training program was to increase the resistance of the future cosmonauts to spaceflight factors. The program was based on up-to-date ideas on these factors, their physicochemical and biological properties and on the individual characteristics detected in the course of examining the candidates.

Special attention was paid to the factors associated with the motion of the spacecraft or with the unusual living conditions in the vehicle.

Of great importance were the nervous and mental stress caused by responsibility for carrying out complicated tasks and by the unusualness of the situation, confinement in a small space with sharp limitation of movements, peculiar microclimate in the cabin, acceleration, weightlessness, vestibular stimulation, etc.

The tandem flight of A. G. Nikolayev and P. R. Popovich showed that the general principles followed in the training program were sound. However, they are far from perfect, and they certainly cannot be mechanically applied to training women.

The standards of physical exercises for women, including competition, are somewhat lower than for men. Yet the parameters of the spaceflight factors are the same for both. Consequently, the female cosmonaut had to receive the same training. It was necessary, of course, to allow for female anatomical and functional characteristics, particularly the change in reactivity at different times in the menstrual cycle. /240

Special experiments were performed on female rhesus monkeys and hamadryas baboons for this purpose. Investigation of the effects of intense accelerations revealed that the smallest changes occurred in the proliferative phase of the ovarial-menstrual cycle, with only slight changes during the ovulation period. These findings led to the formulation of appropriate centrifuge exercises and to the determination of optimum times for women to fly in a spacecraft.

The special cosmonaut training program included flights on aircraft adapted to create brief weightlessness, tests in heat, pressure and soundproof chambers, application of vibrations, whirling on a centrifuge, parachute jumping, general physical and other types of training.

Since weightlessness tends to alter the interactions of the human sensors established under the conditions of terrestrial gravity, the training program aimed at increasing the resistance of all sensors responsible for regulating spatial perception. The purpose was to prevent or diminish the illusions of spatial position and symptoms of motion sickness that might arise during flight. The notion that women are more sensitive than men to rocking motion was not borne out by special studies on the phenomenon.

In general, the training increased the cosmonauts' resistance to spaceflight factors, strengthened volitional qualities and improved the neuropsychic processes.

Radiation Safety

The radiation background during an orbital flight is determined by (a) primary cosmic radiation; (b) radiation from radiation belts; (c) radiation associated with chromosphere bursts on the Sun.

As in the earlier flight, radiation safety was ensured by carefully selecting the orbit, constantly observing phenomena on the Sun before and during the flight and monitoring the radiation background by means of onboard radio meters

as well as individual dosimeters. A variety of biological objects /241
(lysogenic bacteria, drosophilas, certain plant seeds, etc.) was an additional means of checking on the radiation dose. Study of these objects after the flight determined the degree of injury caused by cosmic rays. A comparison of the data showed that the radiation situation during the flight was normal. According to the readings of the individual meters, the total dose received by Bykovskiy and Tereshkova was about 80 and 44 mrads, respectively.

Feeding the Cosmonauts

Feeding arrangements for Bykovskiy and Tereshkova before the launching and during the flight were the same as for A. G. Nikolayev and P. R. Popovich. The onboard food supply consisted of the basic ration called for by the planned duration of the flight plus a reserve for emergencies. The calorie value of the basic ration was 2,500-2,900 kcal/24 hr. The content of the food was in accordance with hygienic norms (90-100 g of proteins, 75-80 g of fats, and 300-350 g of carbohydrates). The amount of vitamins was somewhat increased. The ration included natural foods as well as puree-like substances in tubes. The individual preferences of the astronauts determined the choice of foods. Water was supplied through a special device, which functioned satisfactorily during the flight.

Microclimate in the Spacecraft Cabin

Normal physiological reactions depend to a certain extent on the microclimatic conditions in the cabin: temperature, humidity, oxygen and carbon dioxide content and barometric pressure. The parameters were close to the optimum in the flights of Vostok 5 and Vostok 6.

The air temperature in Vostok 5 ranged from 13-26°, in Vostok 6, 14-24°; humidity was 37-60 and 25-40 percent; oxygen, 22-28 and 24-27 percent; carbon dioxide, 0.23-0.50 and 0.05-0.50 percent. Barometric pressure remained at 760 ± 20 mm Hg throughout the flight. As in the earlier space experiments, the parameters of the microclimate in the cabin were automatically maintained at the prescribed level. Moreover, the cosmonauts were able to regulate /242
the temperature manually if they wished. Throughout the flight they maintained careful visual control over the air regenerating and conditioning system. Oral communications about the functioning of the life-support system were duplicated by telemetry.

Analysis of the telemetry information revealed that the air regenerating and conditioning system functioned very satisfactorily throughout the flight.

Biomedical Methods of Investigation

The many experiments performed on Earth, in aircraft and spacecraft showed that partial and especially complete weightlessness may alter the interaction of the afferent systems and sensors in the integral organism. The resultant disturbances are manifested in various ways, particularly in the form of illusions about spatial position and in reactions resembling the symptoms of motion sickness. The mechanisms of such phenomena have not yet been wholly elucidated.

Consequently, there is need for further study of the causes of such reactions and the mechanisms of their development.

The cosmonaut training program was designed to prevent these phenomena from arising. The cosmonauts were told how to suppress autonomic reactions, should they develop. The entire routine, set of calisthenics and diet were successful in preventing the autonomic nervous system from being adversely affected.

Unnecessary vestibular and optokinetic stimuli were also eliminated. The program of biological measurements on board Vostok 5 and Vostok 6, which were more or less the same as those made during the flights of Nikolayev and Popovich, was aimed at investigating the effects of spaceflight factors on the organism. In both cases, the following were transmitted through the radiotelemetry channels: (1) EEG in the forehead-occiput lead (the electrodes were mounted in the cosmonaut's helmet); (2) cutaneogalvanic reaction with the electrodes placed on the foot and on the lower third of the shin of the right leg; (3) respiratory rate (by means of needle and contact sensors); (4) pulse rate (by means of electrocardiophone).

Several additions were made to the training program for the new /243 flight. These were designed to (1) increase the reliability of communication of biomedical information through the radiotelemetry channels; (2) transmit via telemetry seismocardiograms reflecting myocardial contractility; (3) improve several sensors to record cardiac activity and respiration and methods of placing them on the female body.

Special tests were used to detect latent forms of vestibular-autonomic insufficiency and to study the effect of weightlessness on the sensor system. Some of the tests were designed to reveal potential impairment of coordination of fine movements in case the vestibular centers should be overexcited by spaceflight factors. The other tests made it possible to evaluate the degree of vestibular excitability in response to additional weak vestibular stimulation. In the former, during certain orbits the cosmonaut took various tests (finger-nose, "outstretched hands," "writing" with copying of standard pictures and text). In the latter, a series of movements involving bending and straightening the trunk, turning, tilting and rotating the head, eye movements (optokinetic stimuli) served as additional stimuli of the vestibular apparatus. The results of the tests were evaluated from subjective sensations (presence of dizziness, illusions), autonomic reactions (pulse and respiratory rates, cutaneogalvanic reaction, EKG) and from the somatic (motor) indices--degree of impairment of writing and deflection of the hand and from the nature of the electrooculogram.

Other sources of information, besides the above, for appraising the cosmonaut's health and performance were radio conversations, television observations, the results of physiological, psychological and vestibular tests, the way the logbook was kept, onboard tape recorder and execution of certain elements of the flight plan.

For example, cardiac activity was controlled from the pulse rate and EKG, which reflect the electrophysiological and contractile activity of the myocardium. Respiration was judged from the frequency of the respiratory cycles and its depth; vestibular function, from the electrooculogram, cutaneogalvanic reaction and other autonomic indices; central nervous system function, from the changes in several indices, including EEG data and the cutaneogalvanic reaction. The cosmonauts' efficiency and psychomotor activity were /244 evaluated from a great variety of indices, including analysis of special tests, oral reports, dynamics of movements, facial expression, EEG, cutaneogalvanic reaction, etc.

Results of Physiological Investigations during Flight

A great deal of physiological information was obtained during the flights of Vostok 5 and Vostok 6. To determine the effect of individual spaceflight factors on the human organism, the data were grouped by the four main flight stages: prelaunch, entering into orbit, orbit and descent.

Prior to the launching, both cosmonauts felt well. They actively helped to put on the sensors, take the physiological readings and special functional tests and check the various systems of the spacecraft.

Four hours before the launching, Bykovskiy's pulse averaged 68 beats/min; Tereshkova's, 84. As the blastoff time approached, the cardiac rate of both cosmonauts distinctly increased. In the 5-minute readiness period Bykovskiy's pulse fluctuated between 121-137 beats/min; Tereshkova's, 125-144. The respiratory rate at this time was virtually unchanged--10-24 respiratory cycles per minute. Despite the nervous and emotional strain, the cosmonauts were outwardly calm and very collected, and they followed all instructions precisely.

On entering orbit, the cosmonauts were exposed to a number of factors, the most powerful being acceleration. Their general condition was good. The pulse rate during the first minute of the flight was 154 beats/min and 156 beats/min in Bykovskiy and Tereshkova, respectively.

The cardiac rate gradually decreased and by the end of the active portion of the flight it was slower than during the 5-minute countdown period.

Tereskhova's pulse was more rapid than Bykovskiy's and it was slower in returning to normal. The respiratory rate of both fluctuated slightly, averaging 20 respiratory cycles per minute.

As the vehicle was entering orbit, the cosmonauts carried out the programmed observations and maintained contact with the ground stations. For example, /245 as soon as the nosecone of the rocket was cast off, Tereshkova said: "I see the horizon. An azure, blue band. That's the Earth! How beautiful it is!"

Careful analysis of the physiological data, radio conversations and television observations shows that both cosmonauts readily tolerated the spacecraft's entering orbit and carried out their assignments in full. Changes in the pulse and respiratory rates were caused both by nervous-emotional stress

and by dynamic spaceflight factors such as acceleration, noise and vibration. The important thing is that the reaction of the female organism was not peculiar in any respect at this time.

The change from high gravitation to weightlessness was deemed to be very mild and smooth. Bykovskiy's pulse rate, high at the start of weightlessness, became normal 5-6 hours after the launching, while Tereshkova's approached the original level after 18-20 hours. Tereshkova's pulse rate, unlike Bykovskiy's, subsequently fluctuated considerably.

While the two cosmonauts were sleeping, the pulse generally dropped and remained at 46-60 beats/min.

The pulse distinctly accelerated 5-6 hours before conclusion of the flight, obviously the result of the nervous and emotional excitement associated with the vehicle's entering the dense layers of the atmosphere, ejection, etc. The respiratory rate ranged from 12-25 cycles. The patterns of the seismogram and EKG remained virtually unchanged throughout the flight.

Bykovskiy's EEG in weightlessness exhibited a tendency for high-frequency oscillations to replace low-frequency oscillations (below 8 cps) with a gradual decrease in amplitude of the bioelectric rhythms, whereas in Tereshkova the low-frequency potentials increased.

Neither cosmonaut had pronounced nystagmus. The cutaneogalvanic reaction was more marked in the first half of the flight than in the second.

Analysis of the physiological data reveals that the cosmonauts had adapted to weightlessness. This was confirmed by their oral reports. Bykovskiy said that he felt very well. He enjoyed "floating" around the cabin after leaving the chair. He was reminded of being weightless only when his pencil or logbook floated away.

Bykovskiy remained quite efficient throughout the flight. As called /246
for by the flight program, he piloted the craft manually, maintained radio communication with ground stations and with Tereshkova and observed heavenly and terrestrial orienting points.

In accordance with the program, reports were received from the cosmonauts of the readings of the instruments as well as of their pulse and respiratory rates. Many impressions of what they saw and experienced were recorded in the logbook or on the tape recorder.

Analysis of the television observations showed that the cosmonauts' movements were coordinated and in line with their oral communications. When Bykovskiy freed himself from the chair, he was able to make precise movements and shift around while taking vestibular tests. He had no disagreeable sensations while doing so.

Both cosmonauts completed the flight program. However, their behavior under weightless conditions was different. Tereshkova was much less active. Her

remarks in the logbook and on the tape recorder were less detailed than Bykovskiy's. The differences in their reactions to weightlessness were apparently due to individual characteristics.

The cosmonauts slept and rested during the flight at the times called for by the program. They fell asleep quickly. Their sleep was deep. On awakening they immediately resumed work.

Entry into the dense layers of the atmosphere was attended by acceleration. The pulse rate accelerated markedly at this time. For example, during the first few minutes of the descent Bykovskiy's pulse fluctuated between 90 and 100 beats/min and at the end increased to 132-157, becoming more rapid than when the craft went into orbit. Tereshkova's maximum pulse rate was 184 beats/min.

The cosmonauts were ejected at a low altitude and landed without mishap. They were met by local residents and physicians in the search parties. Medical examination revealed the cosmonauts' health to be very satisfactory.

Thus, the flights demonstrated that spaceflight of up to 5 days for men and 3 days for women is feasible, if the cosmonauts are carefully selected by medical examination and then properly trained. No pathological reactions are likely to occur.

The main physiological functions during the flight were characterized by (a) increase in the pulse and respiratory rates and shortening of the intervals of the EKG and seismogram in the prelaunch period, when the spacecraft went into orbit, before and during the descent to Earth; (b) gradual normalization of the pulse and respiratory rates and lengthening of the EKG, intensified bioelectric activity of the cerebral cortex in Bykovskiy, and appearance of low-frequency oscillations in Tereshkova during the orbital portion of the flight.

The cosmonauts' capacity for work in weightlessness remained at a fairly high level.

Finally, all biomedical systems for checking the condition of the cosmonauts and microclimate of the spacecraft functioned normally.

RESULTS OF BIOLOGICAL INVESTIGATIONS CONDUCTED DURING FLIGHTS OF "VOSTOK" TYPE VEHICLES WITH THE PARTICIPATION OF COSMONAUTS A. G. NIKOLAYEV, P. R. POPOVICH AND V. F. BYKOVSKIY

V. V. Antipov, N. L. Delone, G. P. Parfenov and V. G. Vysotskiy

Under spaceflight conditions, the organism is exposed to a combination of different factors, the least studied of which are weightlessness and cosmic radiation. Investigation of these factors is not only of theoretical interest, but has great practical significance as well. It is probable that weightlessness and cosmic radiation will be the main barriers to the penetration of space by man (ref. 1). /248

Manned spaceflights of several years' duration will obviously become feasible only after the creation of partially or fully closed, reliably functioning ecosystems incorporating organisms of varying complexity. Consequently, it is even now essential to know how weightlessness and cosmic radiation, individually and in combination with other flight factors, affect the basic biological processes: the multiplication, growth and development of organisms; in what directions they may change hereditary characteristics and the physiology of subcellular and cellular structures, and to what degree this will affect the equilibrium of ecosystems. The mechanisms of possible changes must be studied and agents sought for countering them, if they turn out to be harmful.

At the present time it is practically impossible to study the biological effect of these factors under ground conditions, because technological limitations still do not permit the creation of a prolonged weightless state or the production of the individual components of cosmic radiation with the requisite energies. This circumstance makes it necessary to concentrate on the study of weightlessness and cosmic radiation in flight experiments. /249

This report discusses the results of experiments conducted with the participation of Cosmonauts A. G. Nikolayev, P. R. Popovich and V. F. Bykovskiy. The main purpose of these experiments was to study reproductive processes in Drosophila melanogaster under conditions of weightlessness, and also to study the effect of individual flight factors, especially weightlessness and cosmic radiation, on hereditary structures in Tradescantia paludosa.

Let us consider the experiments with Drosophila melanogaster conducted on the Vostok-3 and Vostok-4 spacecrafts. Males and females of the wild laboratory strain Domodevovo-32 (D-32) were used in the experiment. The tests were made in paired plexiglass test tubes connected with a rubber sleeve, arranged so that the tubes could be joined together or cut off from one another (fig. 1).

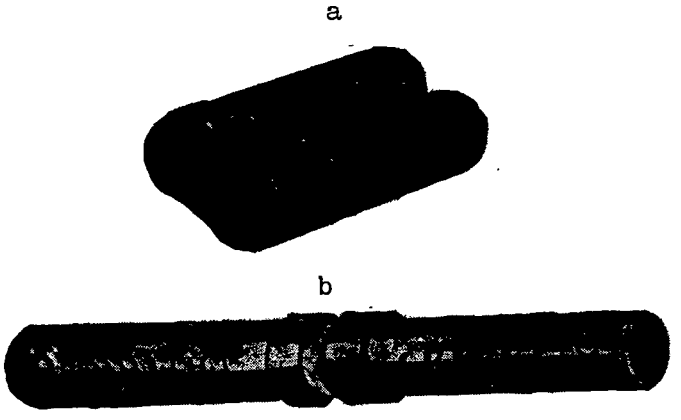

Figure 1. Plexiglass test tube housing during flight. a, Position of tubes before launch and during liftoff; b, position of tubes during weightlessness.

Six hours before launch, 40 males and virgin females 36 hours old were placed separately into the tubes (capacity, 20 ml). The flies were placed on a nutrient base consisting of an 0.8 percent solution of agar-agar and a 10 percent sugar solution with a small amount of ground raisins and fresh yeast.

A. G. Nikolayev and P. R. Popovich each had one pair of tubes. On the first orbit they joined the tubes, permitting the flies to mate. On the 38th orbit (57 hours after launch) A. G. Nikolayev shook down all his flies into one tube. This permitted separate analysis of offspring developing from /250 eggs laid only under conditions of weightlessness. In the other tube (Vostok-3) and in the Vostok-4 experiment, the offspring from eggs laid during weightlessness were distinguished from those laid after landing by the time of pupa formation.

In planning these experiments, it might have been expected that conditions of weightlessness would affect the processes of copulation and oviposition.

It was further supposed that exposure of the developing organism to new environmental factors which had never been encountered in the process of phylogenesis might well result in massive disruptions of morphological organization, of the morphosis type. In addition, it was quite within reason that weightlessness, by changing the action threshold of the genetic system, the phenotypical role of which had never been determined, might produce a Waddington effect.

Finally, during development under weightless conditions the resultant of any polygenetic system might be shifted outside the limits of the field of action of the system controlling the formation of the features in question. In this case, excessive variability due to "genetic scattering" would occur (ref. 2).

Analysis and evaluation of the postulated genetic disruptions were based on the rate of embryo development, on the number, sex and weight of the flies hatched out and on deviations from normal in body shape or size, eye size, color, or shape and size and veining of the wings. Mature sperm from hatched-out males was analyzed for the presence of lethal and visible recessive mutations in the sex chromosome.

The following results were obtained. The flies began to emerge from the pupa on the 11th day after the spacecraft had landed, i.e., on the 15th day after breeding. As is known, under laboratory conditions at a temperature of 25°C the period of development for flies of the strain D-32 is 9 days. The observed 6-day delay can apparently be explained by the development of the embryo and the first larval stages at a temperature around 20°C and also by the unusually "spartan" nutrient medium. The first pupae in the cultures appeared on the 7th day after landing. Therefore the duration of the pupa stage was 4 to 5 days (the normal term for a temperature of 25°C). This normal duration of the pupa stage is indirect evidence that the increased length of the larval stage was caused by low temperatures and not by specific spaceflight factors.

Analysis of the experimental data also showed that the rate of oviposition and the viability of embryos and larvae was approximately the same during weightlessness and after landing. In all cultures the number of females was considerably higher than the number of males, and the latter hatched out one day later (table).

The greater number of emergent females than males may be attributed /251 to several causes. One of these is that spaceflight affects sperm containing Y-chromosomes selectively by making them more difficult to utilize. The skewing of the sex ratio among the later hatching flies might also be attributed to Y-chromosome loss during meiosis. However, it is more probable that under the conditions of the experiment the competitive survival fitness of the male larvae decreased, or that of the female larvae increased.

In addition, some differences in weight were observed between the experimental and control flies. The average weight of the males in the experimental group was approximately 0.2 mg less than for males bred under laboratory conditions. The average weight of the females exceeded that of the males by approximately 0.5 mg in the experimental and 0.7 mg in the control group. Flies whose embryonic and larval development occurs at lower temperatures usually weigh more. In our experiments, however, the flies of the experimental group, which had been kept at lower temperatures on the average than the controls, weighed less. It is most likely that the decrease in body weight can be attributed to raising on a nutrient medium with an increased agar-agar content, although other explanations can also be found.

In the 482 flies obtained in the experiment, four different developmental anomalies were noted. All of these affected only one half of the body and were probably not transmitted by heredity. The anomalies established were /252 absence of half the thorax, unilateral absence of wing macrochaetae, diminution and coarsening of one eye and shortening and malformed veining in one wing.

NUMBER OF FLIES DEVELOPING FROM EGGS LAID DURING SPACEFLIGHT

Time of hatching after landing, in days	Eggs laid during weightlessness		Eggs laid during weightlessness and after landing				Total	
	Vostok-3		Vostok-3		Vostok-4			
	Females	Males	Females	Males	Females	Males	Females	Males
11	14	-	8	-	8	2	30	2
12	55	8	32	4	33	4	120	16
13	10	21	51	24	9	4	70	49
14	-	5	63	45	11	13	74	63
15	-	-	10	25	2	9	12	34
16	-	-	-	-	6	6	6	6
Total	79	34	164	98	69	38	312	170

During examination of hatched flies to discover dominant visible mutations in the autosomes and recessive visible mutations in the sex chromosome of males, not a single indubitable hereditary change of these types was found. However, the analysis was performed on a limited number of chromosomes, which of course does not permit any definite conclusions to be drawn.

Thus, we have reviewed the basic results of the experiments conducted with Drosophila melanogaster on the Vostok-3 and Vostok-4 spacecrafts. These experiments showed that impregnation, oviposition and development of Drosophila can proceed normally under conditions of weightlessness of up to 4 days' duration.

However, these data have only a qualitative significance and do not provide a basis for quantitative evaluation of changes in mating energy, viability of embryos and larvae and the like. These indices might change owing to the geotropism of Drosophila, but in order to observe such changes it would probably be necessary to determine the ± variance of these indices in lines selected by this feature.

In addition, in order to perform quantitative studies it would be necessary to create an artificial overpopulation, as was done in the present experiment. This would obscure changes in the energy of mating and interfere with exact evaluation of the effect of flight factors on the viability of the organism and so forth.

In our analysis of the experimental material, we also failed to observe any essential effect of flight factors on form development in Drosophila. It was indicated that the stunted growth of individuals is fully explained by their being raised on a denser nutrient medium under conditions of overpopulation. However, the percentage (0.83%) of developmental anomalies observed by us was quite high, and therefore the possibility of the predominant elimination of anomalous individuals must be considered. However, their character was nonspecific and their absolute number was not great. The questions, whether spaceflight factors affect the development of forms, and exactly which factors have such effects, must be the subject of further study.

In all probability, the average value of certain indicators may be shifted in one direction or the other when individuals develop under conditions of weightlessness. Total variance, which indicates the exactness of development under various environmental conditions, and individual variance, determining the stochastic variability of development, are also subject to change.

Particularly interesting is the study of phenotypical occurrence of mutations affecting wing structure in the development of mutants, especially under conditions of weightlessness. Environmental conditions which are new in principle are capable of causing, in those mutants where a phylogenetic basis is /253
lacking, the development of forms quite different from those which develop under normal conditions.

Let us proceed to review and analyze the results of experiments conducted on Tradescantia paludosa on the orbital spacecraft Vostok-4 and Vostok-5.

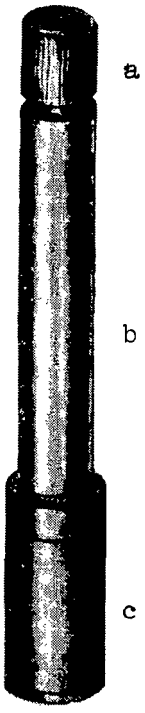

Figure 2. Biocapsule containing cut racemes of Tradescantia during spaceflight. a, Knob of screw closing aperture of fixation fluid reservoir; b, fixation fluid reservoir; c, raceme container.

Cut racemes of Sax clone Tradescantia were placed in special biocapsules, which were designed to permit the cosmonaut to perform bud fixation in flight as scheduled in the program (fig. 2). In the Vostok-4 experiment the racemes were cut 6 hours, and in the Vostok-5 experiment, 9 hours before launching. P. R. Popovich fixated his Tradescantia buds on the 37th orbit, i.e., approximately 57 hrs after launch. V. F. Bykovskiy fixated his experimental materials three times--1.5, 76 and 120 hrs after insertion into orbit. In addition, special specimens were fixated at various intervals after landing (48 hrs after the landing of Vostok-4 and 3.5 hrs after the landing of Vostok-5).

Crushed acetylcarmine preparations were subjected to microscopic analysis. All chromosome disturbances detectable in the Tradescantia microspores were counted, not only those in the metaphase and anaphase, but also those in the telophase. The object and methodology of this study are discussed in greater detail in the studies of N. L. Delone (refs. 3, 4 and 5).

By having the cosmonauts help with these experiments, we hoped to be able to obtain data on the biological effectiveness of individual flight factors; and, naturally, it was of the first importance to differentiate the effects of

factors acting during lift-off and landing of the spacecraft from the effects of weightlessness and cosmic radiation.

Cytological analysis of the microspores of Tradescantia subjected to spaceflight made it possible to observe chromosomic rearrangements, disturbances of the mitotic mechanism and disturbances of growth processes. Thus, in the material fixated by P. R. Popovich, the incidence of chromosome rearrangements was 1.49 ± 0.04 percent, as against 1.89 ± 0.08 percent for the Vostok-3 experiment. Notwithstanding that the Vostok-4 microspores were fixated in a more sensitive phase, the percentage of chromosome rearrangements in the /254 Vostok-3 specimens (fixated 18 hrs after descent) was higher. This can only be explained by the absence of any effect on the microspores of factors occurring during reentry.

The experimental materials obtained with the participation of V. F. Bykovskiy can be divided into the following groups: (1) anthers fixated in flight at 1.5 hrs; (2) 76 hrs; (3) 120 hrs after launch, and (4) anthers fixated 3.5 hrs after landing. A fifth group contains materials from the Vostok-6, fixated 4.5 hrs after landing.

By analyzing metaphase cells in the different groups, it was preliminarily determined that the following phases had been affected. Thus, in group 1, the late prophase and metaphase were affected; in group 2, the late interphase, early, middle and late prophase and the metaphase were affected; in group 3, the early, middle and late interphase and the prophase; in group 4, the early and middle interphase, the prophase, and the late interphase; and in group 5, the early and late interphase and the early and middle prophase.

It should be emphasized that the cells analyzed in the metaphase had been exposed to flight factors during a single postmeiotic mitosis, since the preceding division (in meiosis) had occurred long before this exposure.

Comparison of data for these few experimental groups from the Vostok-5 show the greatest number of chromosome rearrangements to have occurred in group 1; the number decreases in groups 2 and 3 and increases slightly in group 4.

In this comparison, the degree of reliability (P) between the data of the first and second groups is 2.7; between the first and third groups, 4.7, and between the first and fourth groups, 6.1. Consequently, a statistically reliable difference is observed between the number of chromosome rearrangements in the first and third, and the first and fourth, experimental groups (fig. 3).

In the first group, the factors arising during lift-off acted on very sensitive phases and in the third group on less sensitive phases. The fourth group was acted on by factors occurring both during lift-off and landing, which means that different phases were affected.

A relatively detailed analysis of the types of fragments occurring in all four groups showed very few (0.06 percent) spherical fragments in the first group; in later groups their number increases considerably. In the second

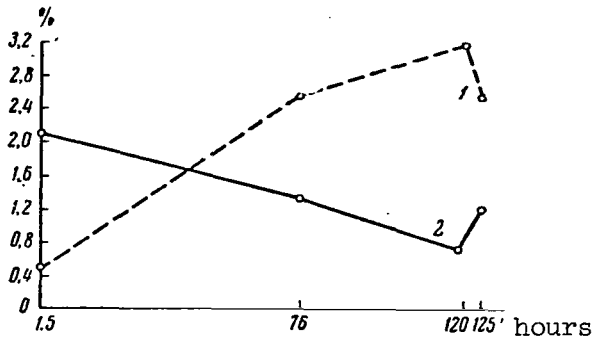

Figure 3. Percentages of chromosome rearrangements and mitotic mechanism disturbances in Tradescantia microspores. 1, Percentage of mitotic mechanism disturbances; 2, percentage of chromosome rearrangements.

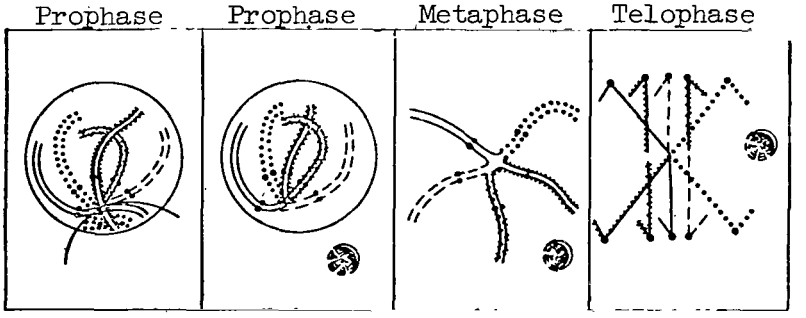

Figure 4. Formation of spherical fragments and chromosome recombinations involving 5 chromosomes.

and third groups there were no chromatid fragments, while in the first group this type of rearrangement was quite frequent. These same regularities were also observed when the various types of chromosome recombination were examined: no chromatid recombinations were observed in the second and third groups, but a considerable number of them were found in the first group. Figure 4 /255 illustrates a possible mechanism for the formation of spherical fragments and complex recombinations.

Four types of disturbances were discovered, reflecting both changes in the regular movement of the nucleus and changes in the mechanism by which mitosis itself takes place (fig. 5).

Study of the numbers of cells with mitotic mechanism disturbances in each group showed that the smallest percentage of pictures showing mitosis disturbances was observed in the first group. The degree of reliability of differences in the data was as follows: between the first and second groups,

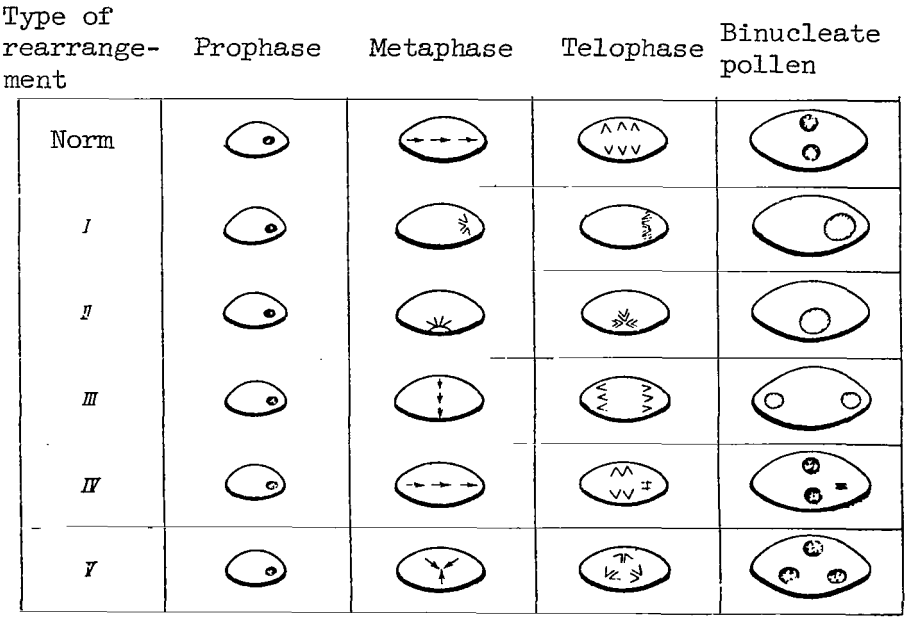

Figure 5. Scheme showing types of mitotic disturbances in Tradescantia microspores.

2.5; between the first and third groups, 10.0; between the first and fourth groups, 10.5. Consequently, a statistically reliable difference was observed between the numbers of cells with mitotic disturbances in the first and third, and first and fourth, experimental groups.

The earliest changes to occur are those in chromosome orientation, following which nonseparations of chromosome complexes begin to appear in large numbers. Delayed nonseparation of chromosomes and tri- and quadripolar mitoses were very rare and were not recorded at all in the first experimental group.

Figures 6 and 7 show microphotographs of spherical fragments in Tradescantia microspores and of cells exhibiting mitotic mechanism disturbances.

We will attempt to analyze the material in order to differentiate the effect of individual factors and groups of factors, and to distinguish those types of disturbances which might be used as criteria in evaluating the effect of these factors on the living cell.

The hypothesis has been advanced that the chromosome rearrangements observed are the result of dynamic factors occurring during lift-off and descent of the spacecraft. At the same time we assume that conditions of weightlessness /256 do not cause the appearance of chromosome rearrangements.

This hypothesis is supported by the following facts. First, the greatest number of chromosome rearrangements was recorded in the experimental material which was fixated 1.5 hours after the launch of Vostok-5, and the smallest /259

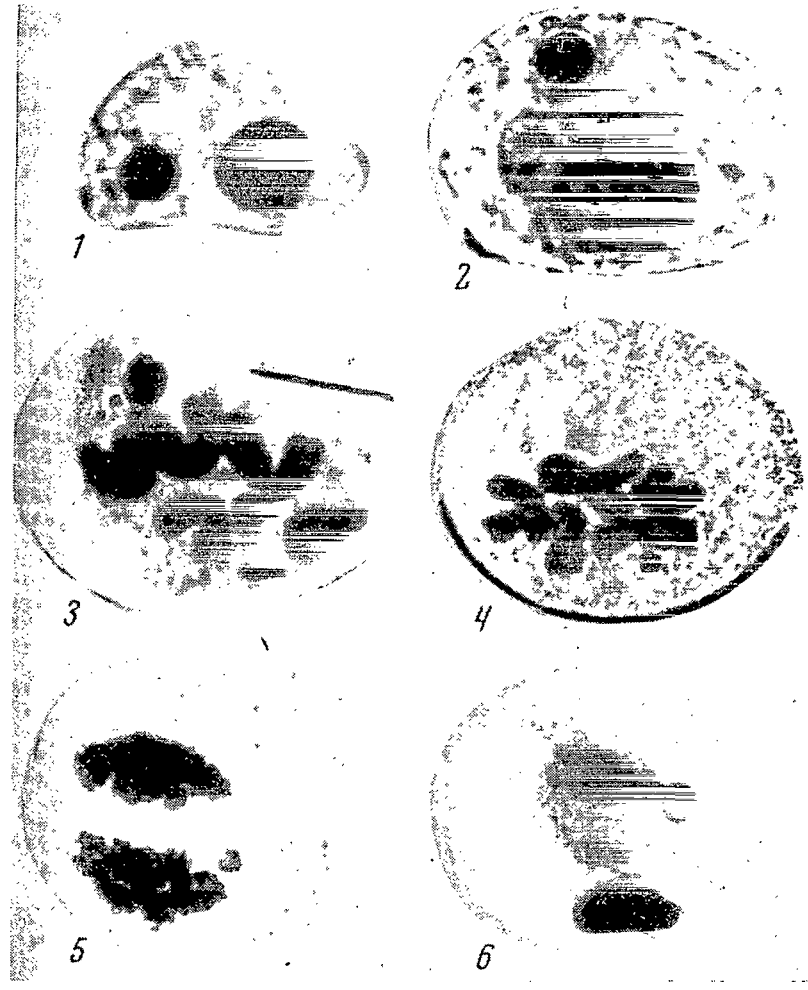

Figure 6. Types of chromosome rearrangements in Tradescantia microspores. 1, Interphase with spherical fragment; 2, prophase with spherical fragment; 3, metaphase with spherical fragment; 4, metaphase with chromosome fragment; 5, telophase with spherical fragment; 6, binucleate pollen with spherical fragment.

number of rearrangements before landing, i.e., 120 hours after launch and 1.5 hours before landing. In the material fixated 3.5 hours after landing, the number of chromosome rearrangements was again observed to increase.

Secondly, it has been shown in laboratory experiments that exposure to ionizing radiation (x-rays, gamma rays and protons) causes chromosome rearrangements to appear considerably later than does exposure to several mechanical factors. With acceleration in particular, chromosome disturbances in Tradescantia spores may occur 5 min after the beginning of the exposure (ref. 6).

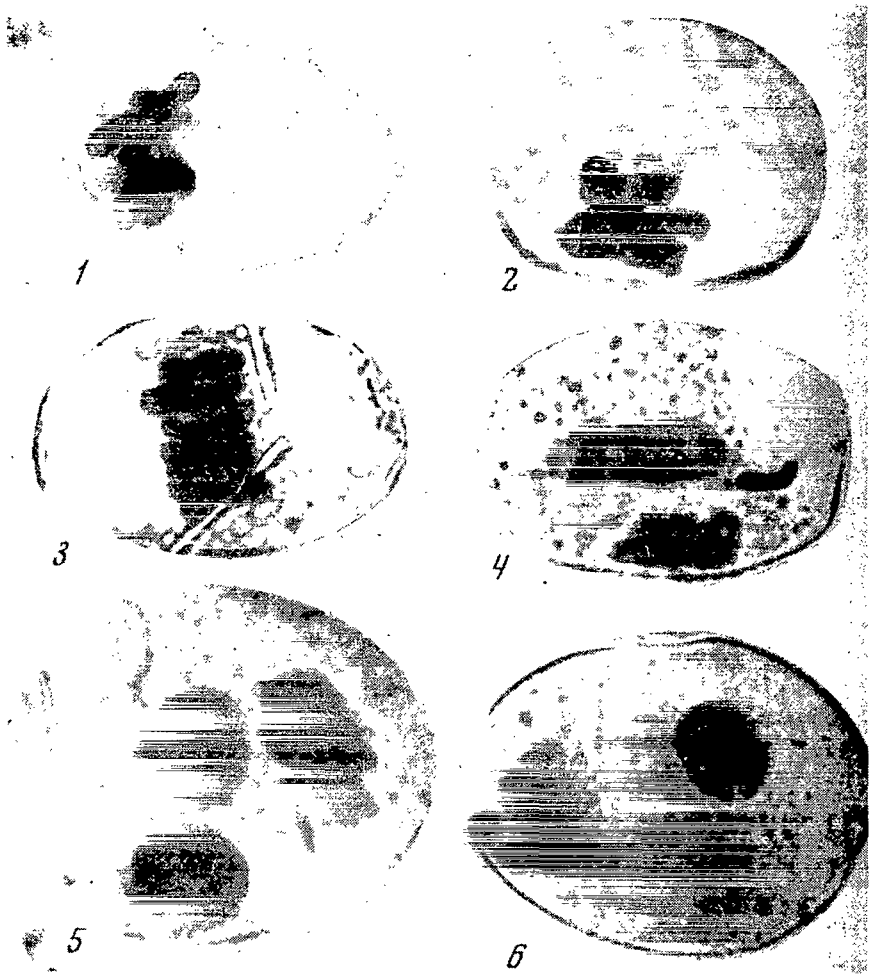

Figure 7. Pictures displaying mitotic mechanism disturbances in Tradescantia microspores. 1, First type; 2, second type; 3, third type; 4, fourth type; 5, fifth type; 6, binucleate pollen with abnormal position of nucleus and spherical fragment.

The third argument supporting this hypothesis is as follows: the action of lift-off factors on the interphase and prophase causes the formation of an overwhelming number of a particular type of chromosome rearrangement--spherical fragments. These same fragments were observed by us after exposure to acceleration ($\sim$ 5,000 g), and in those instances also they were formed when the acceleration acted on the interphase and prophase.

However, when this factor acted on the metaphase, anaphase, and telophase, chromatid and chromosome rearrangements were formed. Analysis of the various types of chromosome disturbances appearing on exposure to flight factors showed that 1.5 hours after launch, a large number of chromatid rearrangements and a

minimal number of spherical fragments occurs. Some 76 and 120 hours later, chromatid rearrangements disappear and spherical fragments predominate.

We cite a fourth argument in support of the hypothesis that factors occurring during spacecraft landing are responsible for the formation of chromosome rearrangements: altogether 5 hours elapsed between the last fixation accomplished by V. G. Bykovskiy and the fixation accomplished after landing, but in the material fixated on the group a greater number of rearrangements was observed. The types of chromosome disturbances once again include chromatid rearrangements.

Consequently, it may be supposed that further increase in the duration of flight, or in other words, prolongation of exposure to weightlessness, will not cause any intensification of genetic effects attributable to chromosome rearrangements.

The data also do not eliminate the possibility that weightlessness may cause disruptions of the mitotic mechanism. What are the facts which support this supposition? First, the smallest number of pictures showing mitotic mechanism disturbances occurred 1.5 hours, and the greatest number 120 hours after launch. Second, factors acting during descent of the spacecraft did not result in any increase in mitotic mechanism disturbances.

However, in evaluating these data and our hypothesis, we must keep in mind that the phases of mitosis which were acted upon have been only preliminarily determined, and that it is their sensitivity to the various factors, as is well known, which basically determines the character and course of the reaction. /260 As has already been indicated, individual cells of Tradescantia microspores flown on the Vostok-3 and Vostok-6 spacecraft exhibited disturbances of the growth processes, in addition to chromosome rearrangement and mitotic mechanism disturbances. The formation of excrescences in cells, giant cells and some instances of disrupted synchrony of microspore development on the anther were observed. It is impossible to say which factors are responsible for these disturbances, since our experimental material is insufficient; consequently it is only possible to state the facts and describe qualitative characteristics.

Thus, we have attempted to analyze the results of flight experiments in which the effect of individual flight factors on reproduction processes in Drosophila melanogaster and the genetic apparatus of Tradescantia paludosa were studied. These biological objects proved to be convenient models for studying the effect of flight factors on the heredity and variability of organisms not only in ground experiments, but also in flight tests where conditions are notoriously fraught with methodological difficulties.

The participation of the cosmonauts definitely contributed to the possibility of studying the biological effectiveness of the mechanical factors occurring during lift-off and descent of the spacecraft and during comparatively lengthy periods of weightlessness. It is quite evident that weightlessness acts in concert with cosmic radiation; however, the total dose of ionizing radiation on these flights was small (40-80 mrad), and consequently few of the

observed effects can be attributed to the action of this factor. However, the degree to which the heavy component of galactic rays has contributed to these effects is not clear.

There can be no doubt that the experimental material obtained and in the hypothesis advanced here require further experimental verification, refinement and confirmation in new flight and laboratory tests. The present studies should be regarded as a starting point for major experiments in future space laboratories.

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RESULTS OF MICROBIOLOGICAL AND CYTOLOGICAL INVESTIGATIONS CONDUCTED DURING THE FLIGHTS OF "VOSTOK" TYPE VEHICLES

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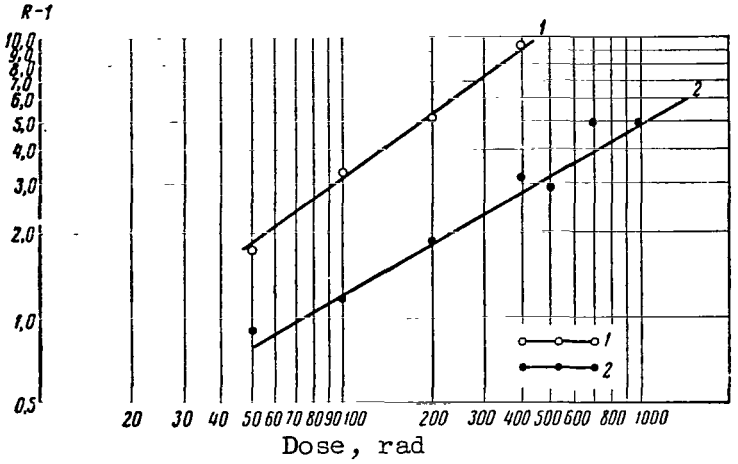
The recent progress made in the study of clonal lines of monolayer /261
cultures of animal and human cells are of great value in solving problems in medical genetics. Specifically, microbiological and cytological objects are being increasingly used to simulate inherited anomalies caused by external mutagenic factors (refs. 4 and 6). Thus, it has been extremely worthwhile to use such models in the study of the "genetic situation" in space, especially in connection with the execution of the program to master space.

The main feature of the biological objects used in the space experiments (lysogenic bacteria and human cell cultures--HeLa strains, amnion and fibroblasts) is that these models served both as biological dosimeters of cosmic radiation and as unique genetic indicators of factors that could cause hereditary changes of pathological nature. One of the better known genetic indicators is the lysogenic culture E. coli K-12 (λ), which by virtue of its great sensitivity to ionizing radiation is a remarkable example of a hereditary anomaly of apparently transformational nature (ref. 4). This is indicated by the mass of factual material obtained in experiments on the biological effect of different kinds of ionizing radiation on the lysogenic strain E. coli K-12 (refs. 5, 7, 8 and 10). It was demonstrated that the lysogenic bacteria had only to be exposed to small doses of gamma rays (of the order of 0.3- /262
0.5 r), protons or rapid neutrons (with a minimum dose of 0.3 rad) for the genetic system of prophage to be irrevocably committed to the formation of infectious phage with a lethal outcome for the bacterial cell. Consequently, phage information is a potentially lethal character resulting in the bacterial cell becoming exceptionally sensitive to mutagenic factors as compared with other well-known biological objects.

The studies of a French investigator (refs. 10 and 11) and the extensive work done in the Institute of Experimental Biology, USSR Academy of Medical Sciences, have revealed that the degree of induction of phage formation by a lysogenic culture is directly related to the radiation dose. It is evident from the illustration (data of N. I. Rybakov and V. A. Kozlov) that the induction curve after gamma irradiation of about 500 r bears a linear relationship to the size of the dose, a significant factor in quantitative expression of the biological effect of penetrating radiation.



Relationship between induction of phage formation by E. coli K-12 (λ) and dose of protons and gamma rays. Ordinate, degree of induction of phage formation (R-1); abscissa, radiation dose; 1, exposure to proton; 2, exposure to gamma rays.

A similar pattern, but with a less sloping biological effect curve, was noted after irradiation of a lysogenic culture with high-energy protons (refs. 5 and 8).

Furthermore, special investigations showed that lysogenic bacteria, /263 even when frozen (-30 to 45°C), are capable of cumulating radiant energy during chronic exposure to Co^{60} gamma radiation at a dose rate of 4 r/24 hours. Table 1 shows that the biological effect of gamma radiation is noticeable even after as low a dose as 0.2 r, although a statistically significant increase in induced phage production as a result of the cumulative effect of ionizing radiation starts with 0.8 r.

Thus, laboratory experiments on lysogenic bacteria were the basis for using this model as a special biological dosimeter of the genetic effectiveness of spaceflight factors.

Starting with the second spacecraft and then on the third, fourth and fifth vehicles, not only lysogenic bacteria were exposed, but also normal and cancerous cultures of human cells (fibroblasts, amnion, HeLa). The experiments showed that on the orbits along which Yu. A. Gagarin and G. S. Titov later completed their historic flight there were no factors capable of impairing the viability and original growth potentiality of human cells in a tissue culture. The level of phage production by E. coli K-12 (λ) in experimental samples did not exceed that of spontaneous phage production by the /264 control (ref. 1).

The data obtained in the experiments on this spacecraft were fully confirmed by the findings on the Vostok (refs. 2 and 3). It is evident from

TABLE 1. RESULTS OF INVESTIGATION OF INDUCED PHAGE PRODUCTION BY THE LYSOGENIC CULTURE E. COLI K-12 (λ) AFTER CHRONIC GAMMA IRRADIATION UNDER FREEZING CONDITIONS (DATA OF N. I. RYBAKOV, S. P. YARMONENKO AND G. F. PALYG).

Dose, r	No. of inoculations	Total No. of phage particles	Average No. of phage particles (M ± m)	Degree of induction of bacteria (R)*	Degree of significance (P)
0.2	12	31	2.6 ± 0.76	1.11	0.76
0.4	12	38	3.2 ± 0.49	1.38	0.42
0.8	12	62	5.2 ± 0.32	2.31	0.0002
3.2	12	131	10.9 ± 1.39	4.84	0.0001
12.5	12	259	21.6 ± 0.40	9.60	0.0001
25	12	387	32.2 ± 0.84	11.85	0.00001
Control	12	27	2.25 ± 0.62	-	-

*$R = N_2/N_3$, N_2, No. of phage particles in the experiment; N_3, No. of spontaneous phage particles in the control; P, statistical significance according to Fisher-Student.

table 2 that the behavior of the immunobiological indices of the cultures of normal and cancerous cells carried on board the spacecraft was essentially the same as those of the cultures kept at the spaceport. This means that the cell cultures exposed on the Vostok did not differ significantly from the cultures left on Earth with respect to the proliferation coefficient, lethality rate, etc., or morphological, antigenic and cultural properties.

Analysis of the samples of lysogenic bacteria following the flight of Vostok 2 revealed that the number of phage particles in the experiment was 1.2 times larger than in the control. Although the difference was slight (and statistically insignificant) as compared with the extent of spontaneous phage production in the control, nevertheless the phage-producing activity of the lysogenic culture E. coli K-12 (λ) carried in space tended to increase.

This was confirmed in subsequent experiments on the spacecraft that made longer journeys. Investigation of samples exposed on Vostok 3 showed that the number of phage-producing cells observed after the vehicle landed was 4.6 times higher than the spontaneous level of phage production.

However, in the lysogenic bacteria on board Vostok 4, the number of phage particles was only 1.96 times higher than the spontaneous background of the /265 control culture left at the spaceport.

Thus, the inducing effect on E. coli K-12 (λ) was more pronounced (2.4 times) in the experiments on Vostok 3 than on Vostok 4. The reason seems to be

TABLE 2. GENETIC EFFECTIVENESS OF SPACEFLIGHT FACTORS ON THE VOSTOKS (EXPERIMENTAL DATA ON LYSOGENIC BACTERIA, NORMAL AND CANCEROUS HUMAN CELLS).

Index	Vostok	Vostok 2	Vostok 3	Vostok 4	Vostok 5	Vostok 6
Degree of induction of bacteria (R)*	1.0	1.2	4.60	1.86	3.67	1.70
Significance (P)	-	0.42	0.002	0.001	0.001	0.02
Flight time, hours	1.5	25	94	71	119	71

*$R = N_r/N_s$, where R is the index of the biological effect; N_r is the quantitative expression of the biological reaction in the experiment; N_s is the quantitative expression of the biological reaction in the control; P is the statistical significance.

that exposure to spaceflight factors was longer during the flight of Vostok 3, as shown by the findings on the biological effectiveness of spaceflight factors on normal and cancerous cells in tissue culture. Specifically, it was found that the reexposed culture of HeLa cells on Vostok 4 and Vostok 6 had a longer latent period of restoration of growth capability than did the cells that were in space just once or served as a control in the laboratory. This difference was particularly evident when evaluating the proliferation coefficient (rate of increase in number of cells grown in relation to the number inoculated), which was almost half that of the HeLa cell culture carried twice into space (on Vostoks 4 and 6), as compared with an intact culture or with a laboratory culture carried once into space on Vostok 4.

These data indicate that spaceflight factors have a cumulative effect on normal and cancerous cells in a tissue culture, although the reasons for and nature of the observed changes are not clear and require further investigation. However, a comparison of the results of the experiments on the lysogenic bacteria exposed on Vostoks 3 and 4 with those on Vostoks 5 and 6 failed to reveal a linear relationship between the biological effect and the time of exposure in space.

Thus, despite the fact that the flight of Vostok 5 was longer, the degree of induction of the lysogenic bacteria exposed on this craft was somewhat lower than in the experiments on Vostok 3.

A similar pattern was observed in the experiments on Vostoks 4 and 6.

Thus, regarding the inducing effect on lysogenic bacteria of flight on the Vostoks 3-6, our experiments show that factors were operating on these flights which increased phage production by the bacteria. Moreover, the slight /266 fluctuations noted in the degree of induction indicate that the inducing factors were similar.

Despite the methodological difficulties connected with the experiments, the results throw some light on the causes of the inducing effect of spaceflight on lysogenic bacteria.

The mean value for the induction of lysogenic cultures on the flights of Vostoks 3 and 5 was ~ 3 units, which is equivalent to the inducing effect of 3.2 r of gamma rays. It was 1.8 units for Vostoks 4 and 6, which is equivalent to the inducing effect of about 0.8 r of gamma rays.

The readings of the physical instruments indicated that these doses exceeded the radiation level during the spaceflights. Therefore, the observed genetic effect must have been due to some other factors that arose during the flights, e.g., weightlessness or such mechanical factors as acceleration or vibration. The latter was the most interesting of all. Acceleration, as previously shown, had no inducing effect. Accordingly, samples of the lysogenic culture E. coli K-12 (λ) were exposed in the laboratory to vibration in a /267 range of frequencies (18, 35, 75, 100 and 700 cps), for 15-30 min or an hour with acceleration of 10 G (table 3). In parallel experiments vibration was combined in various ways with gamma irradiation (100 r) from a source of Co^{60} at a dose rate of 21 r/min (table 4).

Using the familiar method (refs. 7 and 9), we determined simultaneously in control and experimental samples the number of viable cells and phage-producing bacteria. The experimental results were evaluated from the index of induction (R), which is the ratio of the level of induced phage production by a lysogenic culture in an experiment to the amount of spontaneous phage production in control samples.

In table 3, the index R is represented in relative values obtained in several individual experiments with vibration of different frequencies and lengths of exposure. It is apparent from the table that the number of phage particles in the experimental samples did not exceed the level of spontaneous phage production in the control. On the other hand, the index R in several experiments was lower than unity, an indication of slight suppression of spontaneous phage production by E. coli K-12 (λ). It will be noted that the amplitude of the fluctuations in the aforementioned frequency ranges of vibration did not significantly affect the value of R, which fluctuated between 0.8 /268 and 1.1. However, another pattern appeared after the lysogenic culture was exposed to vibration combined with gamma irradiation. Table 4 shows that if the bacteria were exposed to vibration for 15 min, irradiated and then vibrated again 15 min later, the number of induced bacteria was 1.4 times greater than after gamma irradiation alone. This difference was statistically significant to within 0.1 percent.

The inducing action of gamma rays was slightly intensified even after a single exposure to vibration 1.5 hours before irradiation.

The effect of vibration 1.5 hours after irradiation, as in the case of vibration alone, was insignificant. The number of induced particles after a combined action (single exposure to vibration and irradiation) was almost the same as in the samples exposed to irradiation alone.

TABLE 3. EFFECT OF VIBRATION OF DIFFERENT FREQUENCIES ON PHAGE INDUCTION BY THE LYSOGENIC CULTURE E. COLI K-12 (λ).

N_r/N_s	18 cps 15 min	35 cps 30 min	75 cps 30 min	100 cps 15 min	700 cps	
					15 min	60 min
R_1	1.1	1.2	0.9	0.7	0.7	0.7
R_2	0.6	1.3	1.8	0.6	0.8	0.7
R_3		1.1	0.9	0.8	1.0	0.9
R_4		1.3	0.9	1.1	1.0	0.9
R_5		0.6	0.8			
R_6		0.5	1.5			
R_7		0.6	0.5			
R_8		0.7	0.9			
R_9		2.8	1.6			
Mean R	0.85	1.1	1.1	0.8	0.85	0.80

N_r, No. of phage particles induced in the experiment; N_s, No. of phage particles formed spontaneously.

Thus, our findings indicate that vibration by itself does not induce phage production, but it increases the sensitivity of lysogenic bacteria to subsequent gamma irradiation. This is also shown by the fact that vibration does not increase the biological effect of ionizing radiation, if applied after exposure to gamma rays.

Our investigations suggest the existence of a possible relationship between the genetic effect noted in the lysogenic bacteria exposed on the Vostoks 3-6 and the combined action of spaceflight factors, specifically, irradiation and vibration. Vibration seems to sensitize lysogenic bacteria to cosmic radiation. This was an important element in the discovery that cosmic radiation in doses in excess of our methodological capabilities has an inducing action.

Another possible source of the genetic changes may have been weightlessness combined with other spaceflight factors, especially ionizing radiation. However, further study of their biological effect will be required before we can find answers to these important questions. This research will be of great value in implementing the biomedical program planned for prolonged spaceflights.

TABLE 4. RESULTS OF INVESTIGATING THE EFFECT OF A COMBINED ACTION (VIBRATION AND GAMMA IRRADIATION) ON THE PHAGE-PRODUCING ACTIVITY OF E. COLI K-12 (λ).

Type of action	No. of inoculations	Total No. of phage particles X 10^3	Average No. of phage particles in the samples (M ± m)	R	P
1. Vibration - irradiation - vibration	5	12,667	2533.4 ± 34.54	9.0	++0.001
2. Vibration - irradiation	5	9,877	1975.4 ± 8.56	6.9	+++0.01
3. Irradiation - vibration	5	9,009	1808.0 ± 23.10	6.4	
4. Irradiation	5	9,108	1821.6 ± 24.02	6.4	
5. Vibration	5	872	174.4 ± 10.50	0.6	++++0.001
6. Control	5	1,417	283.4 ± 22.70	-	

The index "R" was calculated for each type of action in relation to the control; ++P, obtained by comparing the first type of action with the fourth; +++P, obtained by comparing the second type of action with the fourth; ++++P, obtained by comparing the results of vibration with the control.

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REACTIONS OF COSMONAUTS TO WEIGHTLESSNESS

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Some information was obtained during the flights of the Soviet /270
cosmonauts on the effect of weightlessness on the human body. This article attempts to analyze some of the physiological reactions of the cosmonauts who were exposed to brief (familiarization aircraft flights along Kepler's parabola) and prolonged weightlessness (flights on the Vostok spacecraft).

Reactions to Brief Weightlessness

Brief weightlessness lasting 35±5 sec was produced during an aircraft flight along Kepler's parabolic trajectory. In each flight the cosmonauts were exposed to 3-6 periods of weightlessness ("vertical climbs"). They were either free or secured in a chair.

The following indices were recorded: biocurrents of the myocardium (EKG), arterial pressure, pulse and respiratory rates. Coordination of movements was also studied by means of a "writing test" and work on a special coordinograph. In the "writing test" the cosmonaut wrote an assigned text before and during flight along the parabola of weightlessness. In the investigation on the coordinograph, the cosmonaut at a given signal performed the test, successively making contacts in five receptacles of the apparatus that were arranged in two rows 20 cm apart horizontally and 13 cm vertically. A record was made of the time required to perform a single movement while operating the coordinograph and the time it took to make the contacts. The subjective reports of the cosmonauts were also recorded.

The cosmonauts' impressions of how they felt in weightlessness did /271
not change significantly. There were no signs of spatial disorientation. According to P. R. Popovich, "in going into a weightless state the sensations were different. The body lost its weight, there was an unusual lightness of movements...I felt excellent in weightlessness. I oriented myself normally in the cabin and in space." The other cosmonauts felt the same way. During the first "vertical climb" they were all tense and agitated, but after repeated exposures to weightlessness they became calm.

Precise coordination of movements largely depended on whether the cosmonauts were held secure. If they were held firm in the working space, they had no particular difficulty with the "writing test" or with the coordinograph (table 1).

TABLE 1. DATA ON COSMONAUTS' MOTOR ACTIVITY DURING THE FLIGHT ALONG KEPLER'S PARABOLA (MEAN VALUES).

Cosmonaut	Total duration of program carried out on coordinograph, sec		Duration of "pencil" touching contact of coordinograph, sec	
	On Earth	In weightlessness	On Earth	In weightlessness
Yu. A. Gagarin	5.50* / 4.8-5.96	5.85* / 5.32-6.78	0.36	0.37
G. S. Titov	6.0 / 5.88-6.12	5.82 / 4.52-5.88	0.40	0.38
A. G. Nikolayev	7.18 / 7.0-7.36	6.83 / 6.34-7.32	0.40	0.38
P. R. Popovich	9.32 / 8.16-10.48	9.05 / 8.32-9.78	0.85	0.86
V. F. Bykovskiy	3.16 / 3.04-3.28	3.83 / 3.52-4.14	0.18	0.21

*Numerator - mean values; denominator - limits of fluctuations.

It is evident from table 1 that the cosmonauts worked as rhythmically in weightlessness as they did on Earth. The differences in the time indices were insignificant. Visual inspection showed that the cosmonauts' movements were coordinated, smooth and fairly precise. This was confirmed by analysis of the cosmonauts' movements in taking water and food--removal of tubes with water or food packages from their pockets, opening them up and ingesting them. /272 They managed to eat regardless of whether the food was liquid, semiliquid, or solid. The opposite happened when they moved about freely in the cabin. They were unsuccessful in the "writing test" because the movements of their extremities resulted in their shifting about. Yet here also repeated exposures to weightlessness enabled them to develop skills that permitted them to preserve some stability. Muscular strength of the hands was evaluated from a dynamometer (table 2). The value of the maximum muscular exertion decreased considerably in weightlessness--by 6-12 kg for the right hand and by 4-12 kg for the left. One reason for this may be the decrease in tonic contraction of the skeletal musculature.

As mentioned above, some physiological indices were recorded during flight. The main findings are presented in tables 3, 4 and 5. Before flight the pulse rate of the cosmonauts ranged from 62-77 beats/min. In horizontal flight the pulse accelerated by 6-24 beats in most of the cosmonauts, apparently because of

TABLE 2. DYNAMOMETRY OF HANDS (IN KG) BEFORE AND DURING "FREE SOARING" IN WEIGHTLESSNESS.

Cosmonaut	Right hand				Left hand			
	Horizontal flight	"Vertical climb"			Horizontal flight	"Vertical climb"		
		1	2	3		1	2	3
A. G. Nikolayev	56	54	52	48	54	52	50	47
P. R. Popovich	54	48	50	49	50	48	48	46
V. F. Bykovskiy	58	48	46	48	56	44	46	47

TABLE 3. CHANGE IN PULSE RATE OF COSMONAUTS DURING FAMILIARIZATION FLIGHTS ALONG PARABOLA OF WEIGHTLESSNESS.

Cosmonaut (date)	Background (Earth)	"Vertical climb"	Horizontal flight	Weightlessness, sec				Horizontal flight
				10	20	30	40	
Yu. A. Gagarin (5/27/60)	62	1	75	66	54	54	60	76
		2	68	66	60	60	66	78
		3	72	78	54	60	66	70
G. S. Titov (5/27/60)	77	1	93	72	66	66	--	--
		2	--	--	60	72	--	--
		3	--	--	66	72	72	84
A. G. Nikolayev (6/2/60)	70	1	80	84	72	72	72	84
		2	80	78	66	66	72	82
		3	88	72	66	72	72	88
P. R. Popovich (5/27/60)	70	1	94	72	78	78	--	80
		2	88	78	72	78	--	92
		3	92	78	72	74	84	84
V. F. Bykovskiy (6/29/60)	66	1	66	72	66	72	--	66
		2	66	66	60	60	--	--
		3	--	60	60	60	--	--
V. V. Tereshkova (8/10/62)	72	1	84	96	96	84	--	--
		2	--	78	78	--	--	--
		3	--	78	84	90	--	72

TABLE 4. CHANGE IN ARTERIAL PRESSURE (IN MM HG) OF THE COSMONAUTS DURING FAMILIARIZATION FLIGHTS ALONG THE PARABOLA OF WEIGHTLESSNESS.

Cosmonaut (date)	Background (Earth)	"Vertical climb"	Horizontal flight	Weightlessness, sec				Horizontal flight
				10	20	30	40	
Yu. A. Gagarin (5/27/60)	130/82	1	145/60	140/75	--	--	--	150/70
	--	2	--	150/--	--	--	135/65	150/75
	--	3	145/75	--	--	--	145/70	--
A. G. Nikolayev (9/14/61)	122/69	1	123/79	118/74	--	--	--	123/79
	--	2	138/69	103/54	--	--	--	123/64
	--	3	128/64	113/54	--	--	--	103/59
P. R. Popovich (5/26/60)	120/--	1	140/75	--	135/65	--	--	150/75
	--	2	150/65	150/--	--	135/65	--	--
	110/70	3	150/80	155/60	--	135/65	--	140/60
V. F. Bykovskiy (6/29/60)	155/--	1	160/--	--	--	--	--	155/--
	--	2	155/--	160/--	--	--	--	165/--
	150/--	3	--	--	--	--	--	--

TABLE 5. CHANGE IN RESPIRATORY RATE OF THE COSMONAUTS DURING FAMILIARIZATION FLIGHTS ALONG THE PARABOLA OF WEIGHTLESSNESS.

Cosmonaut (date)	Background (Earth)	"Vertical climb"	Horizontal flight	Weightlessness, sec				Horizontal flight
				10	20	30	40	
Yu. A. Gagarin (5/27/60)	18	1	17	15	15	12	--	18
		2	18	15	18	15	--	18
		3	19	24	24	18	--	18
G. S. Titov (5/26/60)	20	1	18	15	15	18	--	18
		2	14	16	18	12	18	18
		3	19	30	18	18	24	19
A. G. Nikolayev (6/1/60)	14	1	15	21	18	18	--	14
		2	14	15	18	15	--	14
		3	14	15	15	14	--	14
P. R. Popovich (5/26/60)	16	1	18	18	21	15	--	22
		2	20	24	18	18	24	20
		3	17	18	24	24	--	18
V. F. Bykovskiy (6/29/60)	18	1	21	21	21	27	22	18
		2	24	24	24	27	--	18
		3	24	27	24	21	24	18

nervous and emotional tension. The cosmonauts clearly reacted in individual fashion. For example, Popovich's pulse increased markedly (by 26-34 percent), as did Titov's (by 20.8 percent) and Tereshkova's (by 16.6 percent), but Bykovskiy's remained unchanged. In weightlessness the cardiac rate generally decreased, mostly from "climb" to "climb" (table 3). The reason seems to be the development of adaptation-compensatory processes and, in part, decrease in the orienting reaction.

Arterial pressure also changed with change in the cardiac rate. It increased during horizontal flight and decreased in weightlessness, barely /273 remaining above the original level (table 4). Nikolayev was an exception. In weightlessness, especially during the second and third "climbs," his arterial pressure fell even below the original level.

The respiratory rate during horizontal flight remained virtually unchanged in three cosmonauts. It increased slightly in Popovich and Bykovskiy. During the first 10 seconds of weightlessness, the number of respiratory movements increased in most of the cosmonauts, and it became more superficial. Later, despite the continuing effect of weightlessness, respiration slowed to the level observed before the horizontal flight (table 5). /274

In weightlessness the EKG became normal aside from a decrease in the cardiac rate. The amplitude of the P and T waves and the duration of the intervals approached the original values.

The materials on the sensory, motor and autonomic reactions during exposure to weightlessness partly coincide with the results of the investigations of other authors involving subjects under similar conditions.

Thus, it is evident from these experiments with brief weightlessness that the cosmonauts experienced some functional changes in a number of systems with a tendency for them to return to the original level. Individual physiological reactions were detected from the magnitude of the autonomic changes.

Reactions to Prolonged Weightlessness

In the period of orbital flight the cosmonauts were exposed to a variety of factors--weightlessness, radiation, etc., but not in equal degree. Weightlessness was the most unfavorable. Cosmic radiation and the other factors /275 had only a slight effect, because the values were low and scarcely different from those prevailing under ordinary terrestrial conditions.

In evaluating the physiological reactions to weightlessness, we must bear in mind that the cosmonauts experienced considerable nervous and emotional strain, especially in the prelaunch period (table 6).

This kind of physiological change is not specific to spaceflight. It occurs in parachutists before jumping, in athletes, etc. Three types of pregame states are distinguished in sports psychology: "combat readiness," "start fever" and "start apathy." The first is characterized by mild excitation

TABLE 6. CHANGE IN PULSE RATE OF COSMONAUTS (BEATS PER MINUTE) IN PRELAUNCH PERIOD (MEAN DATA).

Time before launching		Yu. A. Gagarin	G. S. Titov	A. G. Nikolayev	P. R. Popovich	V. F. Bykovskiy	V. V. Tereshkova
Several days		64	69	64	58	69	70
4 hr		65	69	72	56	68	84
5-min countdown	5 min	102	104	108	111	133	127
	4 min	106	105	106	114	122	124
	3 min	109	107	113	114	121	144
	2 min	101	107	121	128	126	142
	1 min	123	110	120	124	137	139

combined with a business-like mood; the second, by nervousness, agitation, absentmindedness; the third, by sluggishness, apathy. Analysis of the radio conversations, television observations and activity of the cosmonauts shows that their nervous-emotional state is of the first kind, "combat readiness." It is caused by primary and secondary signal stimuli (outfitting, orders to get ready, etc.). /276

During the change from gravity to weightlessness, Gagarin, Titov and Popovich briefly experienced the illusion of being upside down. The probable cause is the fact that weightlessness disrupts the coordination of the sensors concerned with spatial relations (proprioceptive, vestibular, cutaneomechanical and visual) and causes sensory disturbances (refs. 7-10). Nevertheless, the phenomenon did not interfere with the cosmonauts' performance. They were able to observe the external orienting points and functioning of the equipment and maintain radio communication.

Space experiments also produced a great deal of information about autonomic functions. The cardiac rate of the cosmonauts during orbital flight regularly decreased, sometimes to the level recorded several days before the launch (table 7). Titov's pulse 4 hours before averaged 69 beats/min, Nikolayev's 72, Popovich's 56, Bykovskiy's 68 and Tereshkova's 81. In Nikolayev and Tereshkova it returned to the original level about the middle of the second day of the flight; in Popovich and Bykovskiy at the end of the first day. Titov's pulse remained above the original level throughout the flight due to the experimental conditions and vestibular-autonomic disturbances (table 7). However, return of the pulse rate to the original values did not mean that it was normal, for this /277 index fluctuated considerably under weightless conditions. In a short period of time (and with no visible cause) it ranged from ±10-15 beats/min or more. This was particularly pronounced in Titov, Popovich and Tereshkova. Tereshkova's pulse, for example, accelerated during the 20th, 29th-31st and 35th-37th orbits and slowed markedly during the 23rd, 34th and 39th.

TABLE 7. PULSE RATE CHANGE (BEATS/MIN) OF COSMONAUTS DURING DIFFERENT FLIGHT STAGES IN WEIGHTLESSNESS (MEAN DATA).

Flight stage			G. S. Titov				A. G. Nikolayev				P. R. Popovich				V. F. Bykovskiy				V. V. Tereshkova			
			M	σ	C	% of PL_2	M	σ	C	% of PL_2	M	σ	C	% of PL_2	M	σ	C	% of PL_2	M	σ	C	% of PL_2
5-min ready (PL_2)3			106,0	7,31	6,88	100,0	112,0	7,10	6,34	100,0	117,0	7,55	6,45	100,0	132,0	12,39	9,37	100,0	133	8,52	6,41	100,0
Weightlessness	End 1st day	1	104,0	9,89	9,47	100,0	127,0	6,27	4,94	113,4	104,0	5,36	5,15	88,9	101,0	9,22	9,13	76,5	114,0	6,37	5,61	85,7
		7	82,0	8,72	10,63	77,4	78,0	3,20	4,10	69,6	—	—	—	—	63,0	3,99	6,30	47,7	78,0	3,63	4,65	58,6
	2nd day	13	89,0	9,74	10,92	84,0	88,0	8,30	9,43	78,6	62,0	2,40	3,87	52,9	63,0	2,58	4,08	47,7	95,0	11,03	11,67	71,4
		17	97,0	12,24	12,62	91,5	79,0 (16)	11,30	14,30	70,5	60,0	5,65	9,42	51,3	70,0	7,30	10,32	53,0	68,0	6,47	9,56	51,1
		23					65,0	7,73	11,89	58,0	50,0	4,07	8,14	42,7	64,0	4,72	7,33	48,4	61,0	3,92	6,35	45,9
	3rd day	29					65,0 (30)	2,88	4,43	58,0	59,0	7,44	12,61	50,4	50,0	5,66	11,31	37,9	78,0	9,32	11,96	58,6
		33					63 (32)	6,51	9,57	60,7	53,0 (34)	2,70	5,09	45,2	56,0 (35)	3,42	6,07	42,4	69,0	4,09	5,94	51,9
		39					73,0	8,25	11,30	65,2	56,0	2,98	5,32	47,9	62,0	3,91	6,25	47,0	64,0	11,78	18,43	48,1
	4th day	45					72,0	2,70	3,75	64,3	61,0 (46)	1,22	2,00	52,1	58,0	2,49	4,31	43,9	70,0	2,82	4,04	52,6
		51					67,0	3,74	5,58	59,8					58,0	3,96	6,85	43,9				
		55					64,0	2,55	3,93	57,1					70	5,19	7,48	53,0				
	5th day	61					71,0 (62)	5,66	7,97	63,4					57,0	5,66	9,92	43,2				
		67													61,0	4,86	8,01	46,2				
		71													61,0	4,85	7,95	46,2				
	Begin 6th day	78													61,0	4,44	7,32	46,2				
		81													77,0	5,95	7,77	58,3				

Orbits

Notes: 1. M, arithmetic mean; σ, standard deviation; C, variation coefficient.
2. If there is no information during the individual orbits, the data of the next orbits are cited. The numbers are in parentheses.
3. PL_2 = prelaunch$_2$ period.

TABLE 8. CHANGE IN RESPIRATORY RATE (CYCLES PER MIN) OF COSMONAUTS DURING DIFFERENT STAGES OF FLIGHT IN WEIGHTLESSNESS (MEAN DATA).

Flight stage			G. S. Titov				A. G. Nikolayev				P. R. Popovich				V. F. Bykovskiy				V. V. Tereshkova			
			M	σ	C	% of PL_2	M	σ	C	% of PL_2	M	σ	C	% of PL_2	M	σ	C	% of PL_2	M	σ	C	% of PL_2
5-min ready $(PL_2)^3$			19,61	4,00	20,38	100,0	9,67	1,34	13,86	100,0	15,17	2,65	17,47	100,0	16,83	4,88	28,97	100,0	17,28	2,22	12,85	100,0
Weightlessness	End 1st day	1					13,71	2,86	20,86	141,8	19,14	3,18	16,61	126,2	24,25	5,36	22,12	144,0	20,50	3,11	15,20	118,6
		7	13,60	2,22	16,33	69,4	11,44	2,00	17,48	118,3	—	—	—	—	16,60	2,07	12,49	98,6	21,28	2,63	12,38	123,1
	2nd day	13	16,17	5,34	33,01	82,4	11,00	1,80	16,36	113,8	18,20	2,06	11,32	120,0	18,00	2,71	15,04	107,0	22,50	3,10	13,82	130,2
		17	16,61	3,01	18,14	84,7	11,28 (16)	1,58	14,01	116,6	17,38	3,69	21,23	114,6	19,90	2,08	10,45	118,2	21,88	2,99	13,70	126,6
		23					10,88	3,14	28,86	112,5	16,38	0,82	5,01	108,0	21,25	2,63	12,38	126,3	21,00	1,19	5,69	121,5
	3rd day	29					7,5 (30)	1,73	23,01	77,56	15,00	3,46	23,07	98,9	14,00	2,83	20,20	83,2	21,80	1,48	6,80	126,1
		33					11,1 (32)	2,89	26,00	114,79	15,25 (34)	2,83	18,56	100,5	19,0 (35)	3,26	16,79	112,9	21,81	2,69	12,35	126,2
		39					12,40	2,84	22,90	128,2	14,86	2,70	18,17	98,0	18,54	1,62	8,73	110,2	21,54	1,97	9,16	124,6
	4th day	45					10,6	2,41	22,74	109,6	16,33 (46)	2,60	15,92	107,6	18,43	2,48	35,57	109,5	19,67	5,86	29,81	113,4
		51					13,0	3,24	24,92	134,4					18,30	2,83	15,46	103,7				
		55					11,7	2,78	23,75	120,0					18,25	1,25	6,82	103,4				
	5th day	61					9,75 (62)	2,51	25,74	100,8					17,00	1,41	8,31	101,0				
		67													18,86	2,54	13,46	112,0				
		71													19,17	1,48	7,73	113,9				
	Begin 6th day	78													13,5	2,52	18,64	80,2				
		81													17,54	2,44	13,91	104,2				

Orbits

Notes: 1. M, arithmetic mean; σ, standard deviation; C, variation coefficient.
2. If there is no information during the individual orbits, the data of the next orbits are cited. The numbers are in parentheses.
3. PL_2 = prelaunch$_2$ period.

Statistical processing of the results of the examinations of the cosmonauts (table 7) also showed that the standard deviation (σ) and variation coefficient (C) during orbital flight generally increased considerably above the original level. For example, 4 hours before the launch the variability of Nikolayev's pulse rate had the following characteristics: standard deviation, 3.02; variation coefficient, 4.25 percent. In Popovich, Bykovskiy and Tereshkova, 1.97 and 3.52 percent, 2.15 and 4.63 percent and 2.52 and 3.11 percent. But during the prelaunch waiting period these indices were usually higher in all cosmonauts (table 7).

This variability in the physiological indices is an indication of imperfect neuroreflex mechanisms of regulation (refs. 24-27). It is difficult to tell whether the regulatory disturbances are the result of nervous-emotional strain or weightlessness. The latter is the more likely cause because during the extended orbital flights, when nervous-emotional strain gradually decreased, the variability of the pulse even increased a little.

The data on the respiratory rate were less clear (table 8). All cosmonauts had more respiratory movements, especially at the start of the experiment, than in the 5-minute readiness period. The changes were undoubtedly due to nervous-emotional strain. At the start of weightlessness the cosmonauts reacted sharply to any external stimulus, because they had not as yet adjusted to weightlessness or to being in the spacecraft. Furthermore, the respiratory rate was greatly influenced by the rhythm of the radio conversations which were conducted very rapidly throughout the flight.

There were also definite changes in the EKG. The QT interval was shortened during the 5-minute readiness period at the start and end of weightlessness and somewhat lengthened in the middle (fig. 1). The PQ interval changed in about the same way but less distinctly (fig. 2). Changes in the time indices of the EKG were closely related to the pulse rate: the intervals lengthened when /278 it slowed and vice versa. L. I. Fogel'son (ref. 71) observed the same relationship when examining sick and healthy persons on the ground.

It is evident from figures 3 and 4 that the amplitude of the T and R waves changed unequally. The amplitude of the T wave increased in all cosmonauts during weightlessness over that during the 5-minute readiness period (fig. 3), while the R wave increased only in Nikolayev and in Bykovskiy during the first half of the flight. On the other hand, it decreased in Popovich and Tereshkova and during the second half of the flight in Bykovskiy (fig. 4).

The reason for these changes is obscure because even for ground /279 conditions there is still no explanation of the changes in EKG amplitude indices. They may be caused by change in the electrical axis of the heart and, to some degree, by impairment of the metabolic processes in the myocardium. The differences in direction of the changes in amplitude indices may be partly due to the nature of telemetric recording of biomedical information. Discrete recording introduces the element of error in the measurement of individual parameters, especially the rapid amplitude indices.

Figure 1. Change in duration of QT interval on cosmonauts' EKG. 1, G. S. Titov; 2, A. G. Nikolayev; 3, P. R. Popovich; 4, V. F. Bykovskiy; 5, V. V. Tereshkova.

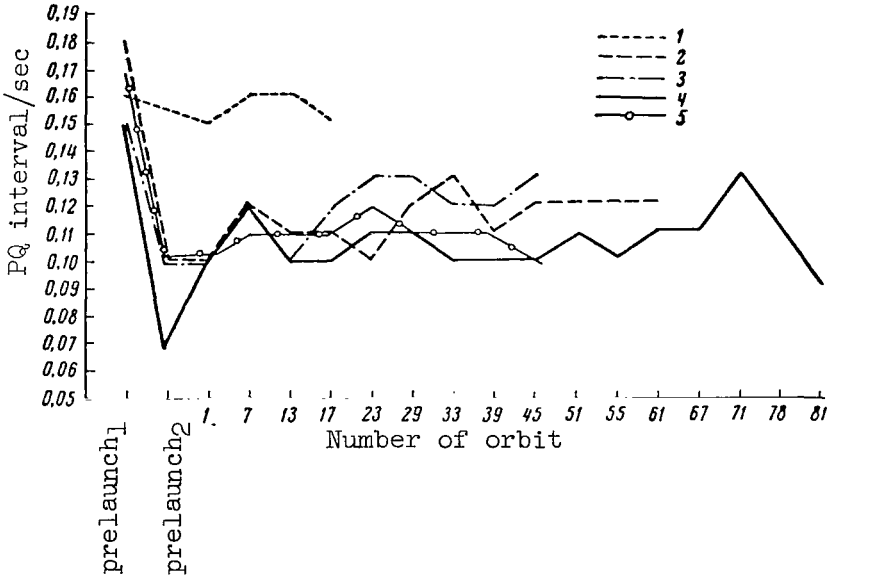

Figure 2. Change in duration of PQ interval on cosmonauts' EKG. Symbols same as in figure 1.

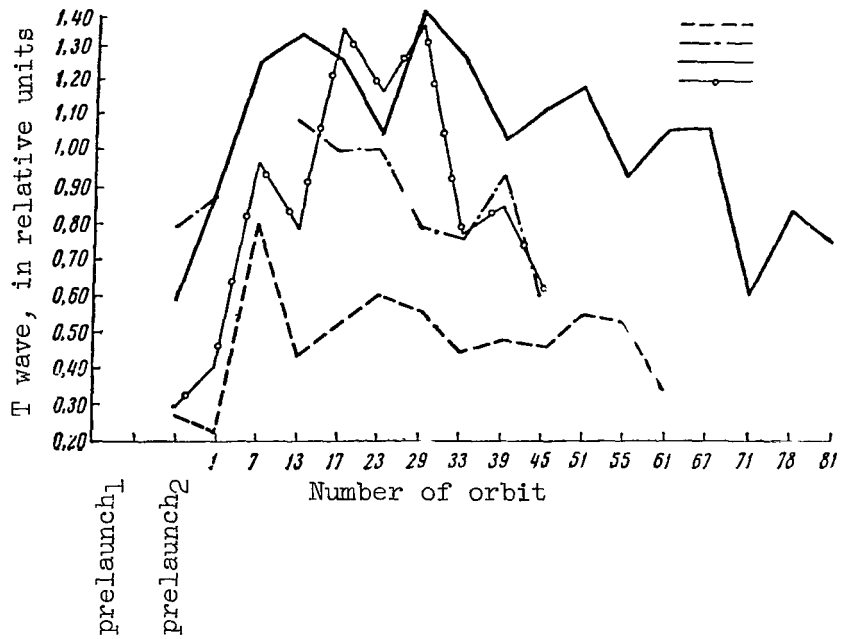

Figure 3. Change in amplitude of T wave on cosmonauts' EKG. Symbols same as in figure 1.

Figure 4. Change in amplitude of R wave on cosmonauts' EKG. Symbols same as in figure 1.

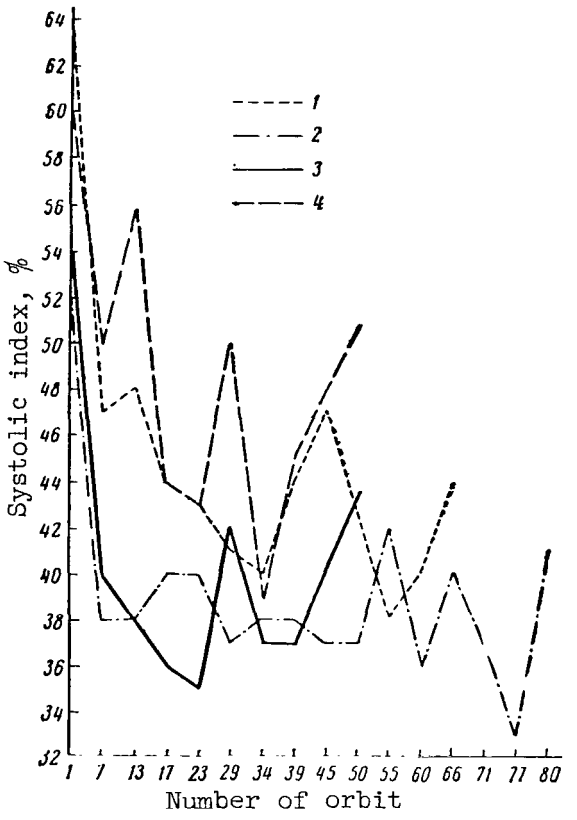

Figure 5. Change in cosmonauts' systolic index. 1, Nikolayev; 2, Popovich; 3, Bykovskiy; 4, Tereshkova.

Experience in interpreting telemetric information has shown that the error in measuring the R wave is greater than in measuring the T wave owing to the difference in duration. /280

The systolic index gradually decreased in weightlessness (fig. 5).

As in the case of pulse and respiratory rates, there was a marked variability in these indices of the PQ and QT intervals, amplitude of the T and R waves and systolic index.

The experimental data suggest that there is no significant impairment during prolonged weightlessness (up to 5 days) except in some indices of cardiovascular function: (1) decrease in cardiac rate, sometimes even below the values recorded on the ground; (2) greater variability in the physiological indices; (3) slow return of some indices to the original level during weightlessness after the active portion of the flight. /281

Some individuals have been found to suffer impairment of spatial orientation in space and to develop the space form of motion sickness (ref. 9).

A comparison of experimental data obtained from exposure to brief and prolonged exposure to weightlessness reveals that the cosmonauts differ in individual resistance to the factor and in the prognostic value of the results of examinations during familiarization aircraft flights along Kepler's parabola. /284 For example, Titov, Popovich and Tereshkova during brief weightlessness exhibited sharp fluctuations in the physiological indices, as occurred during the orbital flights. This underlines the necessity for familiarization flights along Kepler's parabola and taking the experimental materials into account when selecting and training cosmonauts.

We should like to discuss in some detail the mechanisms of action of weightlessness on the human body, since they have been little studied to date. Experimental materials and the literature, in our opinion, indicate that weightlessness acts both directly and indirectly. /285 By direct action we mean the set of reactions stimulated by a marked decrease in body weight (muscles, blood, viscera, etc.). Such a decrease probably lowers blood pressure (arterial and venous), causes blood to accumulate in the venous bed and complicates the venous outflow from the upper part of the body, all of which may give rise to illusions, impair coordination of movements and the like.

Analysis of the experimental material obtained during the long exposure of the cosmonauts to weightlessness shows that the pulse slows, while blood pressure is lowered, sometimes even below the level recorded on the ground. However, it was impossible to establish any definite relationship between a lowering of cardiovascular function and the length of exposure because (1) the autonomic functions are always maintained at a level sufficient for the normal functioning of the major organs and systems (nervous system, skeletal muscles, heart, kidneys, etc.); (2) the impairment of blood circulation caused by the drop in hydrostatic pressure is slight. (According to some authors, blood pressure is not lowered for this reason by more than 10-15 percent); (3) nervous and emotional excitation plays an important role because vagus tone decreases with resultant elevation of blood pressure and acceleration of the pulse rate.

By indirect action of weightlessness we mean the set of physiological reactions stimulated by dysfunction of the sensors (proprioceptive, cutaneomechanical, interoceptive, visual and vestibular) that take part in analysis of spatial relations and position of the body in space (ref. 7). Body equilibrium is known to be ensured by adjusting reflexes, very ancient reflexes, to withstand the force of terrestrial gravity (ref. 8). Under weightless conditions the adjusting reflexes change (ref. 27) because of the significant change in afferent impulses from all the mechanoreceptors. Such changes in the adjusting reflexes inevitably affect the sensory and autonomic components of the reaction. It is easy, therefore, to understand the marked variability of some autonomic indices and the origin of vestibular-autonomic disturbances. The question arises of which mechanism of action of weightlessness on the body is dominant--direct or indirect; when a person is secured, the former seems to be, but when he is not secured, i.e., "floats about" freely, the latter is probably dominant because of the more pronounced changes in the motor component of the adjusting reflexes and the /286 consequences thereof.

The physiological mechanism of the development of the space form of motion sickness appear to be the following: dysfunction of the spatial analyzers, change in reciprocal influences from the otolithic apparatus on the semicircular canals, Coriolis accelerations and conditioned reflex mechanisms (ref. 9), as yet little is known of the reason for the development of this state. A major role in the genesis of the impairment seems to be exclusion of the vestibular sensor because, according to a number of authors (refs. 22, 29, 30 and 42), autonomic reactions are less pronounced in labyrinthectomized animals in weightlessness.

As already noted, some adaptation to weightlessness occurs if the exposure is prolonged. The physiological basis of such adaptation is obviously the formation of new functional arrangements in the work of those sensors involved in analyzing spatial relations and positioning the body in space. This is followed by a strengthening of the autonomic component sufficient to cope with the new conditions.

However, the new arrangements are unstable and likely to be disrupted in the presence of adverse factors (fatigue, temperature influences, etc.). The phenomenon should be kept in mind when planning the work and rest routines of human beings scheduled for prolonged exposure to weightlessness.

We should like to comment briefly on the slow return of the pulse rate in man and animals to the original values under weightless conditions. Some authors have tried to attribute this to the contrasting effects after the organism passes from one state (acceleration) to another--weightlessness (refs. 21 and 36).

Without denying the plausibility of this explanation, we note that the slowness of adaptation seems to be caused both by the length of time it takes for new functional arrangements to take place in the sensors concerned with spatial orientation of the body and by hormonal changes. It is a well known fact that the launching of a spacecraft, acceleration and weightlessness are all powerful stimulants. A large quantity of hormonal substances are released at this time into the blood, where they prevent some indices from returning to normal.

There are two methods of preventing the adverse effects of weightlessness: (1) increasing the specific and nonspecific resistance of the body and (2) improving the equipment of the spacecraft. The former includes measures to strengthen the functional and compensatory capabilities of living organisms by physical conditioning, aircraft flights along Kepler's parabola, use of drugs (refs. 1, 10, 12 and 31) and training the plastic properties of the central nervous system to improve the interaction of the sensors (ref. 9). /287

However, the second approach--technical improvement of the spacecraft--is much more radical and effective. To begin with, the cabin should have optimum physiological-hygienic conditions, which are obviously essential for the normal functioning of vital systems of the organism. The advances in rocket construction in the Soviet Union made it possible to design vehicles in which the prescribed hygienic parameters remain quite stable. Nevertheless, the creation of

optimum physiological-hygienic conditions does not solve the problem, because it does not deal with the fundamental matter of weightlessness. It is necessary to create artificial gravity on the spacecraft, thereby preventing or markedly reducing the unfavorable effects of weightlessness.

Starting with K. E. Tsiolkovskiy (ref. 20), most investigators have maintained that this can be achieved by rotating vehicles. Tsiolkovskiy proposed a spacecraft consisting of two parts connected by a cable with the rotation axis in the center of the vehicle. Calculations showed that a single rotation lasting 11.1 sec (5-6 rpm) creates gravity of 1 g; a single rotation lasting 35.1 sec, 0.1 g (ref. 33). Since Coriolis accelerations act on subjects when they move their heads during rapid rotation (refs. 2, 34, 37, 38 and 40), the rate of rotation of the spacecraft should not be too high. According to Graybiel et al. (refs. 34, 37 and 38), the optimum speed is 1 rpm, since Coriolis accelerations in this case are subthreshold and readily tolerated by subjects. Mayo (ref. 41) thought that to achieve 1 g the radius of rotation of a spacecraft at a speed of 1 rpm should be 900 m.

Ye. M. Yuganov, P. K. Isakov, I. I. Kas'yan et al. (ref. 24) attempted to determine the minimum effective value of the artificial gravity required to maintain normal body position and coordination of movements in animals (white rats and mice). Using a small centrifuge (arm radius 0.9 m) during brief weightlessness (25 sec), they created accelerations ranging from 0 to 1 g. They found that the artificial gravity arising at accelerations of 0.28-0.3 g /288 is the minimum effective value sufficient to prevent the unfavorable effect of brief weightlessness on the motor reactions of animals.

Clearly, one of the most urgent matters calling for experimental verification is the effective value of artificial gravity during prolonged manned spaceflights.

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SOME NATURAL IMMUNITY FACTORS AND COSMONAUT AUTOFLORA DURING THE TRAINING PERIOD AND FOLLOWING THE FLIGHTS OF "VOSTOK," "VOSTOK 2," "VOSTOK 3" AND "VOSTOK 4"

O. G. Alekseyeva

Experiments that we performed jointly with A. P. Volkova (ref. 1) /290 showed that orbital spaceflights and flights on geophysical rockets launched vertically cause moderate wavelike changes in the immunological reactivity of animals, which may alter the autoflora during the first two weeks after landing. Activation of phagocytosis of intestinal bacilli by neutrophils proved to be the most consistent and clear-cut indicator of immunological changes in the "canine cosmonauts." It arose two to three weeks after landing and lasted 1-1/2-2 months.

This finding led to the inclusion of periodic analysis of immunological reactivity in the program of medical observations of cosmonauts.

In choosing methods of immunological investigations, we were guided by two considerations: (1) the examination should take only a minimum of the cosmonaut's time and not injure him, and (2) it should provide a fairly complete idea of the state of the antimicrobial functions of the body and the nature of the autoflora.

We thought that the methods used in radiation immunology would best satisfy the second requirement. Some of the methods were slightly modified to conform to the first requirement. Three groups of tests were used:

(1) The state of the body's bactericidal properties was judged from the lysozyme content of the saliva, intensity of bactericidal properties of the skin and plasma and digestive power of the neutrophils. /291

(2) The phagocytic function was studied in vitro with neutrophils in relation to coccal and rod-shaped microorganisms and in vivo with phagocytes of the oral mucosa in relation to the autoflora. A cytogram of the oral mucosa was prepared, and the intensity of the adsorptive properties of the epithelium was determined.

(3) The state of the autoflora was judged from the quantity, species composition and hemolytic properties of the pharyngeal microflora, surface of the epidermis and deep layers of the skin of the forearm.

These tests took 12-15 min of the cosmonaut's time. The lysozyme content was determined by the usual method with Fleming's lysing micrococcus obtained

from the L. A. Tarasevich Control Institute. Saliva was diluted in 0.5 percent sodium chloride solution (1:100 to 1:51,200). The dilution in which distinct clarification occurred after 2 hours of incubation at 37° C was taken as the titer.

The bactericidal properties of the skin were studied by N. N. Klemparskaya's method (ref. 3) in our modification. A one-day-old broth culture of E. coli was replaced by an agar culture, which is more transportable. The culture was washed with physiological solution to a density of 5 units of turbidity by the Control Institute's optical standard, i.e., to approximately 500 million microbial cells in 1 ml. The suspension was diluted before use to 1:20,000. These large dilutions made it possible to reduce the time of investigation sharply. After the inner surface of the lower part of the left forearm was smeared, impressions were made on plates with Endo's medium 20-30 sec and 1, 3 and 5 min later.

This modification of the method also enabled us to take into account changes in the bactericidal properties of the skin with respect to the microbial cells most sensitive to it. These cells die on the skin within minutes or even seconds. If more concentrated suspensions are used, as Klemparskaya recommends, it is possible to trace only the dynamics of dying of the more resistant individuals which die 30-45 min after striking the skin.

In investigating the antimicrobial properties of the oral mucosa, we used impressions (on slides) with buccal mucosa. The impressions were fixed with methyl alcohol for 3 min and stained by the Romanovsky-Giemsa method. To determine the cytogram, 100 cell elements were counted and the percentage of epithelial cells, intact and altered (with cytolysis and karyolysis) leukocytes counted. In the same preparation we also calculated the percentage of epithelial cells that adsorbed microbes of the autoflora and the /292
percentage of intact leukocytes that participated in vivo in phagocytosis of the oral microflora.

The phagocytic properties of the neutrophils with respect to Staphylococcus albus (Lepin strain obtained from L. A. Tarasevich Control Institute) were studied by the usual method in our modification (ref. 4). In investigating phagocytosis of intestinal bacilli (E. coli strain 675, obtained from the Control Institute), we used Berman and Slavskaya's principle (ref. 2), which is of value in appraising both the ability of neutrophils to engulf microbes and the bactericidal properties of plasma.

The first step in investigating E. coli was the same as in the case of the staphylococcus. We mixed 0.03 ml of 5 percent sterile citrate, 0.1 ml of blood and 0.03 ml of a suspension of a one-day-old agar culture in a sterile isotonic solution (staphylococcus in 10 units of turbidity, E. coli in 5 units of turbidity). Incubation was for 30 min at 37°C. Smears were fixed and stained by the May-Grünwald and Romanovsky-Giemsa methods. The investigation with the staphylococci ended at this point. However, from a mixture with E. coli other smears were made on meat-peptone agar poured into Petri flasks, which were incubated for 2 hours more at 37°. Impressions were then made from the agar on slides, which were fixed and stained the same way as the smears.

One hundred neutrophils were counted in each smear and impression. This was followed by calculation of the percentage of active neutrophils (percentage of phagocytosis) and average number of phagocytized microbes for each active (phagocytic number) and counted (phagocytic index) neutrophil. Live undigested bacteria were found in giant forms in the impressions, a phenomenon that enabled us to distinguish them from the dead forms, which retained their original size, and to determine the percentage of killed digested microbes both phagocytized by the neutrophils and found in the intercellular spaces, i.e., in the plasma.

In studying the microflora in the throat, we used the principle of measured gargling developed by K. I. Turetskiy and Ye. I. Olen'yeva (ref. 5). The throat was gargled with 5 ml of sterile physiological solution. The different dilutions of the washing were then inoculated in a volume of 0.1 ml on Petri dishes with 5 percent blood agar. After 24 hours of incubation, the number of colonies was counted on the dishes, the species of the microbes determined and the hemolytic properties of the cultures noted. The number of microbes in 1 ml of washing was calculated.

The microflora of the skin on the inner surface of the right forearm was studied by the method of impressions on plates with 5 percent blood agar. To supplement the previously described methods (ref. 4), which permit only the flora of the surface of the epidermis to be investigated, we introduced the idea of making impressions after treating the skin with a wad of cotton dipped in a sterile 0.25 percent solution of aqua ammonia. This removed the /293 accumulated flora and encouraged the flora of the deep layers of the skin to come to the surface. Study of this flora is quite valuable, because it is an intimate part of the body and because it reacts to change in the bactericidal properties of the skin sooner than does the flora on the surface of the epidermis.

The investigations were usually conducted in the morning at basal metabolism. The cosmonauts were occasionally examined during the day or after eating. In the latter case, appropriate corrections were introduced into the evaluation of the antimicrobial properties of the oral mucosa and state of the pharyngeal flora.

Results

We thought it worthwhile to distinguish 5 periods according to the physical and mental loads on the cosmonauts:

(1) period of preflight training;

(2) period of prelaunch examinations of the copilot (3-4 weeks and 3 days before a flight);

(3) period of prelaunch examinations of the first pilot (at the same times as for the copilot);

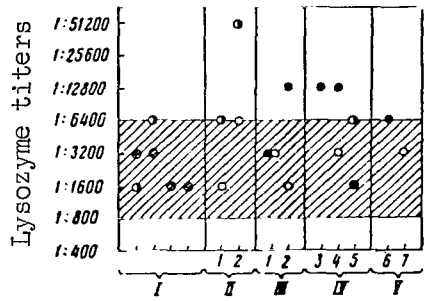

Figure 1. Lysozyme content of cosmonauts' saliva. I, preflight training; II, prelaunch period of copilot; III, prelaunch period of first pilot; IV, first two weeks after flight; V, remote period after flight. Arabic numerals on abscissa indicate days of examination: 1, 3-4 weeks before flight; 2, 3 days before launching; 3, 7th day after landing; 4, 10th day; 5, 13th-14th day; 6, 71st-72nd day; 7, 310th day after flight. Crosshatched field designates boundaries of physiological norm. Indices of Yu. A. Gagarin are indicated by black circles; G. S. Titov's, by white circles; A. G. Nikolayev's, by half-black circles; P. R. Popovich's, by crosshatched circles.

(4) period of examinations during the first two weeks after landing (on the 7th, 10th and 13th-14th days);

(5) period of long-term examinations after flight (on the 71st or 72nd and 310th days).

None of the cosmonauts had an infectious disease throughout the observation period. /294

Let us discuss the results of the studies on the bactericidal properties. Figure 1 is a diagram showing the lysozyme content of the cosmonauts' saliva. It is clear from the diagram that during the preflight training period the values of the lysozyme titers were within physiological norms. During the prelaunch period the lysozyme content gradually increased in both the first pilots and copilots. Three days before the launching Gagarin and Nikolayev (when he was Titov's backup) showed a distinct increase in the lysozyme titer. Lysozyme was activated the first few weeks after the flight only in Gagarin.

Figures 2 and 3 illustrate the study of the bactericidal properties of the skin. This function was highly pronounced in all cosmonauts. In Nikolayev and Popovich it was activated in the training period; in Gagarin, during the

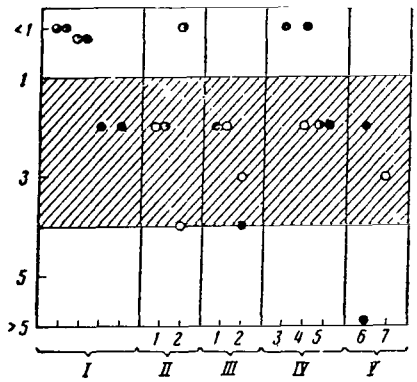

Figure 2. Intensity of bactericidal properties of cosmonauts' skin. Ordinate, time that 50 percent of intestinal bacilli applied to skin died. Other symbols same as in figure 1.

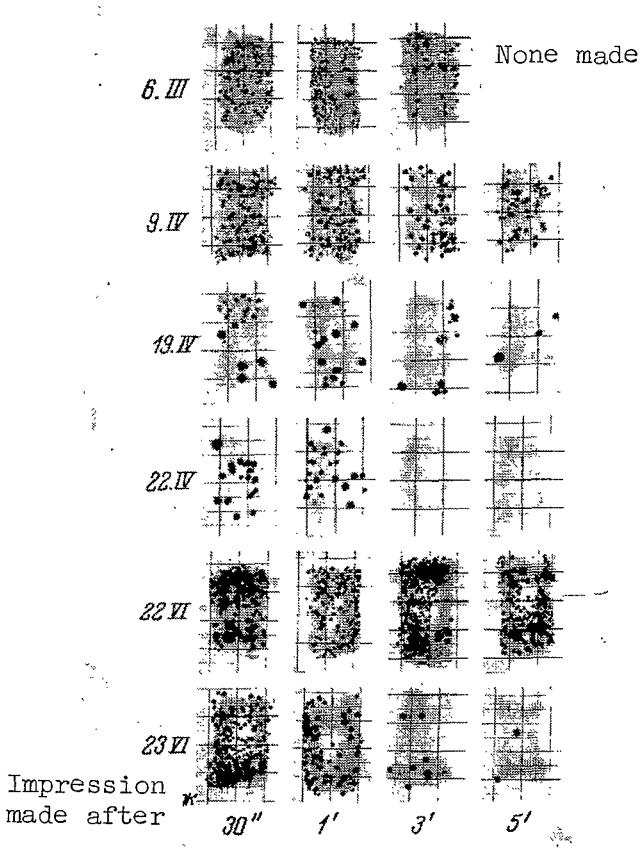

Figure 3. Photograph of impressions used in determining bactericidal properties of skin of Yu. A. Gagarin's left forearm.

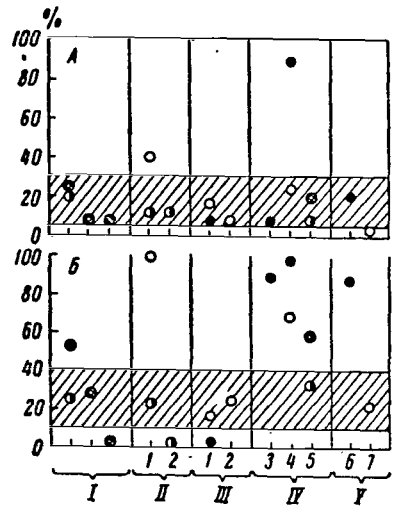

Figure 4. Intensity of bactericidal properties of plasma (A) and digestive function of neutrophils (B) in cosmonauts. Ordinate, number of killed bacteria (in percent). Other symbols same as in figure 1.

first few weeks after the flight, but this activation gave way to lability of the function two and one-half months after the flight. In Titov the bactericidal properties of the skin were more moderate than in the other cosmonauts.

The bactericidal properties of the plasma and neutrophils, as shown in figures 4 and 5, were more stable during the preflight and prelaunch periods; they were normal in the great majority of investigations. The digestive function of the neutrophils became activated after the flight in Gagarin, Titov and Popovich. In Gagarin, it was associated 10 days after landing with an /295 increase in the bactericidal properties of the plasma.

Thus, the bactericidal properties of the body were generally within physiologically normal limits in all four cosmonauts throughout the observation period. Less commonly, they were activated, and in a few cases low. Activation of all indices after the flight occurred only in Gagarin, while only the digestive function of the neutrophils became activated after the flight in Titov and Popovich. After the flight the bactericidal properties /296 remained within normal physiological limits in Nikolayev (fig. 6).

Analysis of the cytogram of the oral mucosa failed to reveal any consistent or significant changes in this index. The ratio of leukocytes to epithelial cells at basal metabolism was stable for each cosmonaut. After eating, the reaction was physiological. The first half hour was marked by local leukocytosis (Nikolayev, 14th day after landing), which was followed 1-1/2-2 hours later by a relative decrease in the number of microphages (Popovich, the same time).

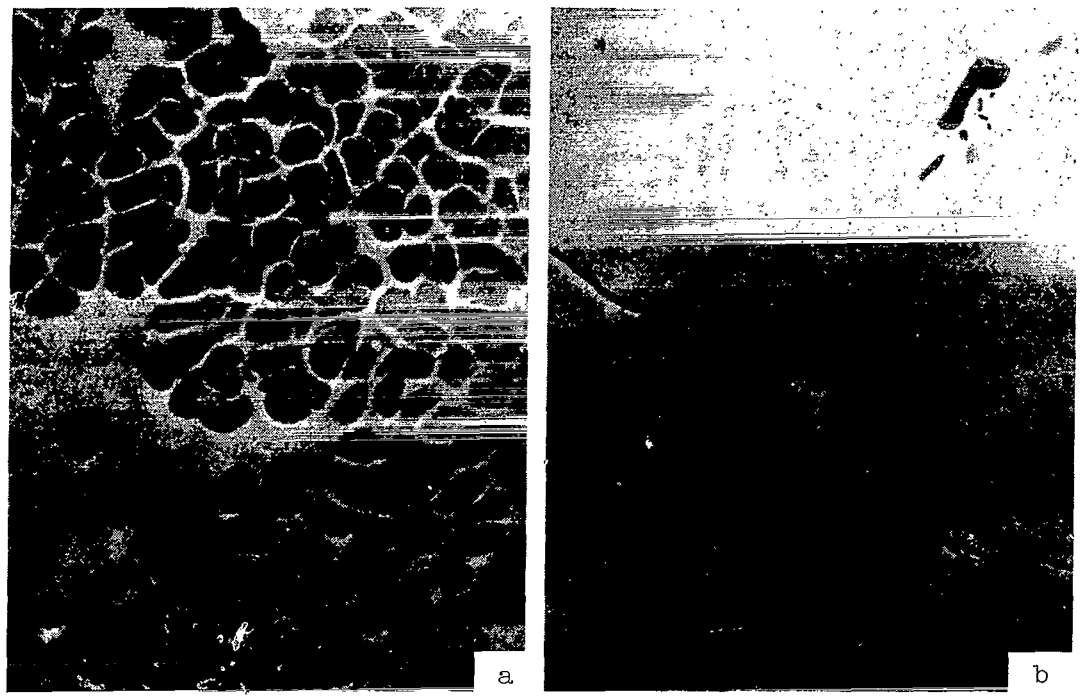

Figure 5. Microphotograph of impressions made during phagocytosis of intestinal bacilli by neutrophils in Yu. A. Gagarin on 10th day after flight (a) and in A. G. Nikolayev on 13th day after landing (b). Dead bacteria are small and gray; live, black. Photograph shows mass of dead bacteria and fragments of neutrophils as well as two neutrophils intensively phagocytizing and digesting bacteria.

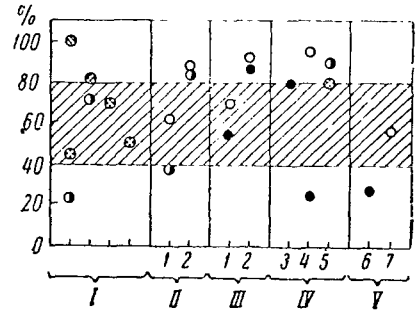

Figure 6. Intensity of in vivo adsorption of cosmonauts' autoflora on epithelium of oral mucosa. Ordinate, epithelial cells adsorbing microbes (in percent). Other symbols same as in figure 1.

Investigations of the antimicrobial properties of the cellular elements in the oral mucosa disclosed that freeing the mucosa in vivo from the microflora always took place rapidly. Figure 6 shows the state of the adsorption properties of the epithelium whose intensity was often above the average physiological norm in all the cosmonauts. The low level of adsorption properties of the epithelium noted in Gagarin after the flight should be regarded not as an inhibition of antimicrobial function, but as the result of impoverishment of the oral flora due to activation of lysozyme and phagocytosis in vivo, especially since adsorption became activated 3 days before the flight, just as it did in Titov and Nikolayev.

Phagocytosis of the microflora of the oral cavity by leukocytes /298
(figures 7 and 8) was activated in Gagarin and Titov even before the launching, and it remained high the first two weeks after the flight. In Popovich, on the 14th day after landing, this index was at the upper limits of normal and higher than during the preflight training period. The activation of phagocytosis in vivo before the "launching" was also noted in Nikolayev when he was the backup pilot. But even in the preflight training period phagocytosis was in- /299
tense. After the flight, however, this reaction was within normal physiological limits.

We will now examine phagocytic activity in blood neutrophils.

Phagocytosis of Staphylococcus albus varied in intensity from cosmonaut to cosmonaut (figs. 9 and 10). In Gagarin, this function became activated the first weeks after the flight, but by the 7lst day it gave way to marked inhibition. In Titov, moderate activation was noted in the prelaunch period and on the 10th day after the flight. In Nikolayev, phagocytosis was generally

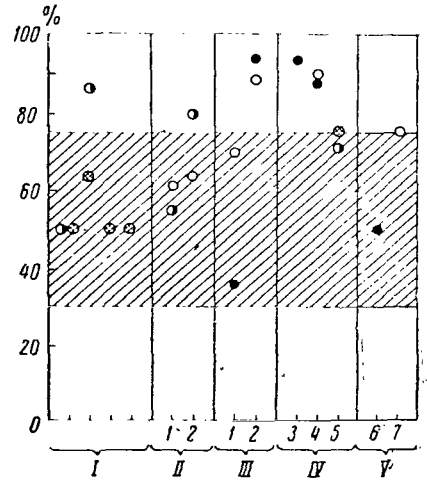

Figure 7. Intensity of in vivo phagocytosis by leukocytes of oral cavity in cosmonauts. Ordinate, leukocytes with phagocytosis (in percent). Other symbols same as in figure 1.

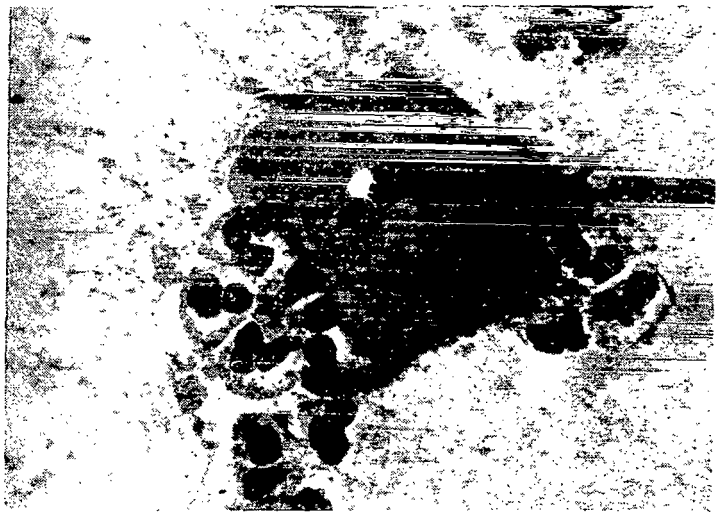

Figure 8. Microphotograph of impression from oral mucosa of Yu. A. Gagarin 10 days after flight. Center, epithelial cell; periphery, leukocytes with phagocytized microbes of autoflora.

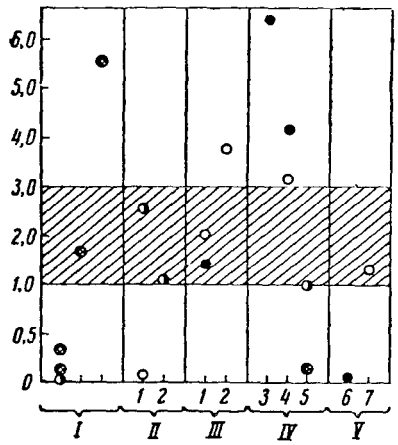

Figure 9. Intensity of phagocytosis of Staphylococcus albus by neutrophils. Ordinate, value of phagocytic index. Other symbols same as in figure 1.

within physiologically normal limits. In Popovich, the process gradually intensified during the training period, but it became very inhibited on the 13th day after the flight.

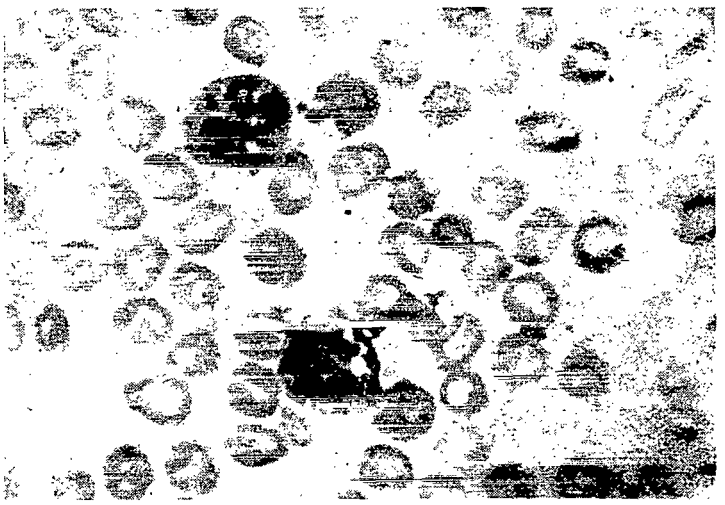

Figure 10. Microphotograph of smear made during phagocytosis of S. albus by neutrophils in Yu. A. Gagarin on 7th day after flight, showing two neutrophils with phagocytized cocci.

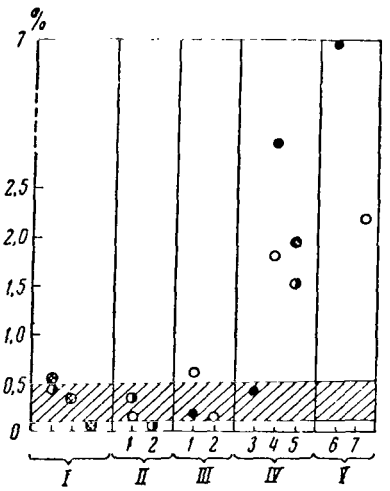

Figure 11. Intensity of phagocytosis of intestinal bacilli by neutrophils in cosmonauts. Ordinate, value of phagocytic index after secondary growth on agar. Other symbols same as in figure 1.

Figure 11, which presents the results of a study of the phagocytosis of intestinal bacilli by neutrophils, shows a distinct relationship between the

intensity of this immunological reaction and the observation period. Adsorption of this microbe started on the 10th day after the flight in all four cosmonauts and it continued for a long time. It will be recalled that in all cosmonauts except Nikolayev it was associated with activation of digestion of the /300
phagocytized bacteria (fig. 4).

The number of microbes in the throat and on the skin of all cosmonauts in the training and preflight periods was within physiologically normal limits. Washing from the throat (1 ml) contained from $2 \cdot 10^8$ to $2 \cdot 10^9$ microbes. Some 2-3 (in cool and moderately warm weather) to 30-50 (in hot weather) microorganisms were removed in an impression made from 1 cm^2 of skin before treatment with aqua ammonia; after treatment of the skin in any weather, from 3-5 to 20-30 bacteria. The increase in number of microbes on the epidermis in hot weather during intensified perspiration was physiological. The number of microbes in the deep layers of the skin was more constant.

The skin microflora was represented by epidermal staphylococci, microbes from the streptococcal group, a few sarcinae and some spore-forming aerobes from the anthracoid group. Thus, the population was quite varied, and the number of epidermal staphylococci frequently totaled 40-60 percent. The hemolytic properties of the epidermal staphylococci were generally pronounced, but the other species of microbes for the most part had no hemolytic capacity.

The throat microflora of the cosmonauts before flight consisted of tonsillar diplostreptococci, streptococci, usually heavily admixed with S. albus, less commonly, with S. aureus. The flora usually had no hemolytic /301
properties, although viridans streptococci were present, generally constituting 5-10 percent of the total streptococci and only once amounting to 63-68 percent in Titov and Nikolayev.

Thus, the composition and properties of the autoflora of all four cosmonauts remained more or less within normal limits during the preflight training period.

After the flight the throat and skin autoflora of Nikolayev and Popovich showed no unusual features. There was a temporary (only on the 9th day) increase in the number of microbes in the deep layers of the skin. The increase in number of nonhemolytic epidermal staphylococci was moderate--double the number three days before launching. In Gagarin there was a relative increase in the number of S. viridans in the throat, 35-51 percent, during the first two months after the flight.

The sharpest changes, although transient, were noted after the flight in the autoflora of Titov. On the 10th day after landing, the number of microbes in the throat was 100 times greater than three days before the launching,

$5 \cdot 10^{10}$ in 1 ml of washing, i.e., approximately 10 times higher than the /302
physiological norm. The number of microbes on the epidermis was unchanged, but it rose 12-fold in the deeper layers of the skin of the forearm over that observed in the earlier investigation, i.e., 9 times higher than the physiological norm. The increase in autoflora was accompanied by dysbacteriosis. The usual throat flora was almost entirely crowded out by gram-negative

nonhemolytic diplococci. Although decomposing sucrose before forming acid, the diplococci did not decompose lactose or mannite and in broth produced turbidity with a slight crumblike sediment. They formed small, convex, lusterless, grayish colonies on blood agar. In the deep layers of the skin, the number of ordinary epidermal staphylococci remained normal. The increase in number of microbes was caused by the appearance of masses of hemolytic micrococci. The micrococci decomposed mannite and sucrose before forming acid, but did not decompose lactose. They produced delicate turbidity in broth and on shaking a "cloud," characteristic of enterococci. On blood agar they formed small semitransparent grayish colonies.

In summary, these immunological changes were transient and, for all practical purposes, insignificant, because they did not result in weakening the cosmonauts' resistance to microbes. The fact that these changes were much less pronounced in Nikolayev and Popovich indicates that cosmonauts can /303 adapt during a 3-4 day flight, and that signs of stimulation either do not appear at all or they are compensated by the end of the flight.

We wish to express our deep appreciation to Ye. A. Fedorov and F. D. Gorbov for help in arrangements.

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UTILIZATION OF HIGHER PLANTS AS INDICATORS OF THE EFFECTS OF ORBITAL SPACEFLIGHT FACTORS ON THE LIVING CELL

N. L. Delone

One of the tasks of biologists engaged in space research is to find /304
models in which to study the effects of spaceflight factors on the living cell.

Although the available physical dosimeters have been highly perfected, there is an unquestioned need of biological indicators. None of the physical dosimeters can indicate the relative biological effectiveness of the action of little studied or completely unstudied factors that may arise in spaceflight. Moreover, the combined action of several factors usually results in a nonadditive intensification of changes in the living organism. In fact, the simultaneous action of several factors often has a different effect from that of each factor separately. It is impossible to find a unique "biological dosimeter." Suitable objects probably have to be selected for each case. We made a search for models among the higher plants, investigating the following species: Allium cepa, Vicia faba, Pinus silvestris, Triticum durum, Triticum vulgare, Lactuca sativa, Sinapis alba, Zea mays, Cucumis sativa, Licopersicum esculentum, Daucus carota, Haplopappus gracilis, Trillium vulgare and Tradescentia paludosa.

Our approach was cytological, i.e., we fixed some portions of tissue of a given plant, stained them and made preparations. Under a light microscope we studied the impairment of various cell structures resulting from the action of different factors.

Chromosomal aberrations, impairment of mitosis and impairment of /305
growth processes in the cell served as the indices.

The aftereffects of the observed changes may be either hereditary (genetic) or transient. The main categories of hereditary changes (mutations) are: point (gene) mutations, chromosomal aberrations, nonbreakage of individual chromosomes resulting in the appearance of hyperploids and aneuploids and nonbreakage of intact chromosome sets resulting in polyploidy. Only the first category cannot be observed cytologically, but the other three categories are suitable for cytological analysis. However, we succeeded in tracing the development of point mutations in such plants as peas, tomatoes, corn and barley not by cytological, but by genetic means, i.e., by noting in the offspring changes in the phenotype.

We recommend the following objects as "biological" dosimeters: dry seeds of certain plants, tradescantia microspores, tissue culture of horse bean and haplopappus roots, bulbs of the common onion, and corn, pine and trillium pollen.

Let us discuss the advantages and shortcomings of each of these objects and tentatively indicate the cases where each can be most effectively used as a "biodosimeter."

Air-dried seeds of higher plants are a most convenient model in radiogenetic investigations. The genetic effect is determined from the number of chromosomal aberrations in the cells of roots of sprouts (in mitosis) or in germ cells (in miosis). The genetic effect can also be appraised from the number of mutant forms in the generations. The nuclei in meristematic cells in seeds are in the "resting" state. There is no division in these cells and the stages of the nuclear cycle are relatively similar, a fact which is helpful in analyzing the cytological material. The peculiar state of these nuclei results in the accumulation of a genetic effect over a comparatively long period of time.

These circumstances as well as the prolonged viability of seeds, convenience of transportation and storage and fairly complete cytological knowledge of several species make dry seeds a basic model for analyzing the residual effects of comparatively long spaceflights on the living cell. However, seeds are comparatively insensitive and they have only limited use (refs. 1-6).

The development of tradescantia microspores represents cell development in the first postmiotic mitosis. The individual phases in the development /306 of the microspores are long (at 20°C the cycle takes 10 days; at 30°, 7 days). In six anthers of one bud the phases occur more or less simultaneously. In one anther, at a temperature of 30° the development of some cells may lag by 12 hours; at 20° there may be an interruption of 18-20 hours in the development of certain cells. The haploid nucleus consists of 6 large chromosomes, which are analyzed in the metaphase. Since tradescantia microspores are in the process of mitosis, they are highly sensitive to external influences. On short flights (no more than 10 days), or if the material can be fixed at certain times during the flight, tradescantia can be one of the best plant biodosimeters and yield a mass of information for cytologists to interpret.

Portions of the roots have dividing cells. The process is mitotic. Since these are highly active cells, they should be sensitive to any external factor. Metaphase or telophase cells are easy to analyze cytologically. Chemical substances dissolved in water enter the roots easily, so that this object can be used in experiments to test various "protective substances."

A tissue culture of roots is conveniently transported, because roots in a nutrient medium can be readily sealed in ampuls. The medium may be liquid or solid and the roots can be fixed in any position desired.

The chromosomes of horse beans have been thoroughly studied. They are large with a diploid number of $2n = 12$. They can be analyzed cytologically both from telophase and from metaphase plates. If the roots are not repassaged, the mitotic index, which remains for some time on one level, drops sharply at the end of the period. Therefore, the time in which a root culture can be used is limited. We performed a special experiment using theophyllin and found that the mitotic cycle in normally dividing cells takes 30 hours at $20 \pm 1°$.

In haplopappus, which has a very small number of chromosomes (2n = 4), both pairs of chromosomes differ sharply from each other. One pair has shoulders of the same size, while the other has one large and one small shoulder and a satellite. A tissue culture can be easily prepared from the roots of this plant.

The use of root tissue cultures as a "biodosimeter" should be very valuable in testing the effect of "protective" substances on living cells during spaceflights.

The common onion has large chromosomes (2n = 14). The cells in the stem of the bulb are at relative rest. However, their sensitivity to external /307 factors is much higher than germ cells in seeds. Consequently, while possessing the same advantages as seeds, they are, in addition, more sensitive. Bulbs can be transported very easily. Onions are recommended on long flights as being more sensitive objects than seeds.

In cytological analysis of pollen, one other test in addition to those described above can be used--the germination of the pollen, which simplifies the analysis considerably. The pollen of some plants can be stored a long time. A good deal of pollen can be collected from corn, trillium and pine. To study the effect of the heavy component of cosmic radiation, the can should be covered with a sensitive emulsion. This method is superior to the other method of analyzing seeds that we used: (1) the number of sensitive units increases because pollen is much smaller than seeds, and (2) the method of cytological analysis is simplified. It is a well-known fact that air-dried seeds of some higher plants and Tradescantia paludosa have already been used by Soviet investigators for these purposes on the flights of the various Vostoks (refs. 1-8).

There is no doubt that the list of higher plants suggested as "biodosimeters" will increase with time.

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PART III. RESULTS OF TEST STAND AND LABORATORY TESTS ON ANIMALS

EFFECT OF PARTIAL RESTRAINT OF MOTOR ACTIVITIES ON BASIC PHYSIOLOGICAL PROCESSES IN MONKEYS

I. D. Bogina, N. A. Rokotova, Ye. S. Rogovenko and R. L. Sheykin

The effects of partial restraint of motor activity on basic physiological processes, behavior and the functional state of the nervous system have been studied in monkeys at our laboratory for the past two years. /308 Such study was found necessary because under the conditions of prolonged spaceflights, partial restraint of men and animals becomes the normal mode of existence for the largest part of the flight.

From data existing at the present time (refs. 1-4) we know that if the monkey is fixed only at the time of the experiment for purposes of measuring his blood pressure, recording his respiration rate and pulse, or taking blood or gastric juice for study, the results of the first tests prove quite heterogeneous. In monkeys there are wide fluctuations in the values characterizing this or that reaction, which disappear only after a considerable number of experiments when the animals have become accustomed to the restraint procedure.

Data published by a group of American scientists (refs. 5-7) on observations of monkeys which had made flights on ballistic rockets showed that quite severe restraint, entailing 24-69 hours in a "straitjacket" condition, did not bring about substantial changes in body temperature, respiratory frequency or heart beat. EKG and EEG data also showed no serious deviations from normal. It must be noted that the monkeys were constantly being trained for conditions of severe restraint. /309 The animals' training began with short tests, which were then extended, as the animal proved ready, to 24 hours and more. However, the authors remark that they did not succeed in accustoming all monkeys to conditions of severe restraint. Some animals proved unsuitable for further testing by clearly manifested fear, increased body temperature and accelerated pulse and breathing.

Lilly and Mason (refs. 6 and 7) observed monkeys in partial motor restraint. They placed the animals in special restraint seats, where they lived from 4-6 weeks to 14-15 months. According to the authors, the monkeys adapted well to partial restraint and by the fourth or fifth day were quite at home in their new living quarters. Prolonged restraint in a sitting position caused the animals to develop some weakness in the muscles of the abdominal wall and edema of the lower extremities.

At the beginning of our own tests we used the restraint chair devised by Lilly and Mason, but later we employed a more convenient and portable restraint device which we built in R. L. Sheykin's laboratory.

The chair developed by the American authors is a cumbersome plexiglass device in the shape of a cube, in which the torso and the pectoral girdle of the monkey are confined. The head is passed through an opening on the upper side of the cube and is fastened by blocks around the neck. The pelvis and lower extremities protrude through an opening in the bottom of the cube, to which the chair is attached. The body of the monkey is restrained by abdominal blocks, and the lower extremities rest on foot rests. The upper side of the cube has openings for the arms, through which the monkey takes food and brings it to his mouth. Under such conditions it is difficult for the monkey to perform such natural actions as peering about and combing himself.

Sheykin's restraint device is based on a different principle. The animal is held by a belt and collar, both fastened to vertical bars clamped on a common base. Depending on the type of monkey, either a seat (for macaque) or a footstool (for capucins) is fastened to the bars. A cuvette-shaped table is set before the animals for food. In these conditions, the animal may move his extremities freely and easily change position. During prolonged fixation in Sheykin's device the monkeys manifested no abdominal muscle weakness and no edema of the posterior extremities.

At the beginning of our restraint experiments with monkeys, we set /310
ourselves the task of following the effects of prolonged partial inhibition of motor activity on the monkeys' circadian behavior rhythm, stimulability by food, and the appearance of postural reflexes. Results of the first series of experiments, conducted by I. D. Bogina, O. P. Bolotina, T.M. Kucherenko, N. A. Rokotova, Ye. S. Rogovenko and R. L. Sheykin on four monkeys, showed that only in the course of the first two to four days of restraint did the animals exhibit slight reduction in the length of sleep and inhibition of the postural-investigatory reflex. Food stimulus, determined from the amount of food consumed daily, remained high during the entire restraint period (10 days to 3.5-4 months), and toward the end of the experiment the animals gained weight. Daily checks of the health of the monkeys uncovered no pathological deviations.

After the animals were freed from the restraint device they experienced certain difficulties in walking: they alternated their lower extremities unsurely and sometimes stumbled. When sitting on a bench, they tried to adopt the posture they had held during restraint: letting their legs hang down and holding on to the bars of the cage. After long restraint (four months) some disturbance in spatial orientation was observed, manifested in inability to judge distance while jumping from one bench to another or from a bench to the wall of the cage. Either the monkey landed short or crashed into the wall of the cage. But all these sequelae were of short duration, disappearing after two or three hours. One male who spent 3.5 months in the chair was mated to a female several days after being freed. His sexual function was preserved intact. The fruit of this union was entirely viable and at the time of this writing is growing and developing.

Continuing our experiment, we sought to confirm that animals who spend a long time in conditions of restrained motor activity can serve as fully reliable subjects for the study of other factors acting on the organism during spaceflight. For this it was necessary to follow not only the general behavior of the animals in restraint, but also to study certain of their physiological processes--the levels of their indices, limits of variation and stability.

The problem set for the second series of tests was study of the circadian respiratory rhythm, heart activity, and bioelectric activity of the brain.

Three monkeys were used in the experiments, two capucins and one macaque. In this second series the restraint devices of Sheykin's design were used, /311 equipped with attachments necessary for recording breathing, motor activity, EKG and EEG.

Objective recording of the respiration rate and motor activity of the two capucins was accomplished by an instrument specially designed for this purpose by Sheykin. It consists of a writing tape drive, two identical amplification and recording channels, a time-mark device and an electromagnetic time relay.

Respiration rate was recorded by pickups sewn into a special vest made of elastic material, which affords the monkey's torso complete freedom. The pickups, in the form of thin rubber tubes filled with carbon powder, were placed in three rings around the monkey's torso. All three pickups were connected in parallel to one arm of a bridge circuit. The potential obtained was amplified by a monocascade dc amplifier. The amplifier output was connected to a low-resistance pen-writing recorder wired to provide pen deviations proportional to the strength of the signal.

The motor activity of the animal was recorded by a second, similar channel from carbon pickups connected to movable parts of the restraint device (the fastenings of the collar and belt by which the monkey was held).

The data were recorded on 35-mm paper tape with time marked at 2-sec intervals. Tape speed was 3 mm/sec. A clock relay was used to switch the device on and off at set intervals. Motor activity showed up on the respiratory record only when the animal made violent movements, which for practical purposes did not disturb recordings of the respiration rate.

Respiration was recorded around the clock in the course of 1, 2, 3 and 15 days of restraint. The average respiration rates and standard deviation were computed from 7 am to 5 pm, 5 pm to 10 pm and 10 pm to 7 am of the following day. This time division was expedient, because the monkeys were housed in the laboratory and were subject to more external stimuli during the working day than at night. Besides, we wanted to compare waking and sleeping respiratory rhythms.

For the most part, the data showed changes in respiration rate to be associated with motor activity. Figure 1 presents the respiration rate of the animals over the first three days of restraint (fig. 1A) and for a 24-hour period following the second week from the beginning of the experiment (fig. 1B).

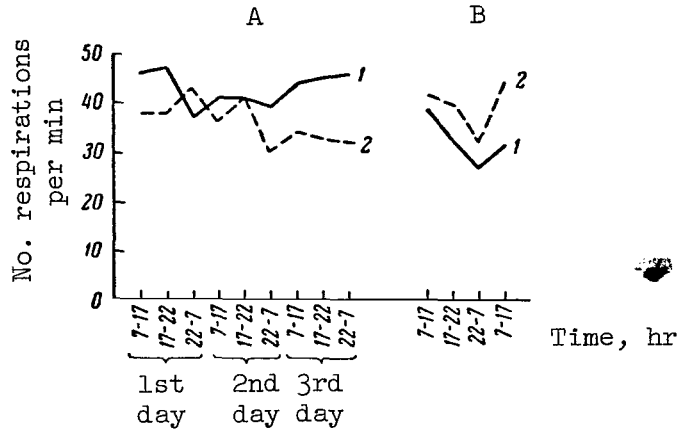

Figure 1. Respiration rate of fixed monkeys. A, respiration frequency in the first three days of fixation; B, after two weeks' fixation; 1, respiration rate of the capucin "Caroline"; 2, respiration rate of the capucin "Yurkiy."

As is evident from the graph, the respiration rate of the monkeys during restraint varied from 32-47 per min. A certain reduction in the rate occurred during /312 sleep. Records taken two weeks after the beginning of the test showed no substantial differences from those made at the outset. Figure 2 presents tracings made by a monkey named Caroline in daytime hours, when the monkey was awake, (fig. 2A) and at night, when she was asleep (fig. 2B). Respiratory frequency in the sleeping animal had a slower rhythm and less amplitude.

Cardiac activity (pulse rate) was monitored through EKGs recorded simultaneously with the bioelectric activity of the brain. The EKG was recorded by plate electrodes with a cutaneous contact in the middle third of the forearm. Cerebral biocurrents were taken off by bipolar parietal and occipital needle electrodes inserted subcutaneously on the right side before each recording. These times the monkey was subjected to more complete restraint by a special plexiglass holder in the shape of the animal's head, with openings for the ears and a soft packing around the edges. The holder was fastened to the restraint device and caught the monkey's head on both sides, preventing him from moving it. After the electrodes were affixed (needle and plate) the restraint and monkey were placed in a screened and darkened chamber, where the animal stayed 35-40 min. The EKG and cerebral bioelectric activity were then recorded. A rhythmically flickering light before the eyes (5, 10 and 15 cps) served as an external stimulus.

As these investigations showed, the pulse rate at rest fluctuated from /313 120-160 beats/min in the macaque and from 200-250 in the capucins. During ocular stimulation the pulse rate rose somewhat, in the macaque, for example, to 200. Over the entire restraint period the pulse rate did not change substantially.

The bioelectric activity of the cerebral cortex in the monkeys can be characterized as follows. The macaque at rest manifested an alpha-like rhythm

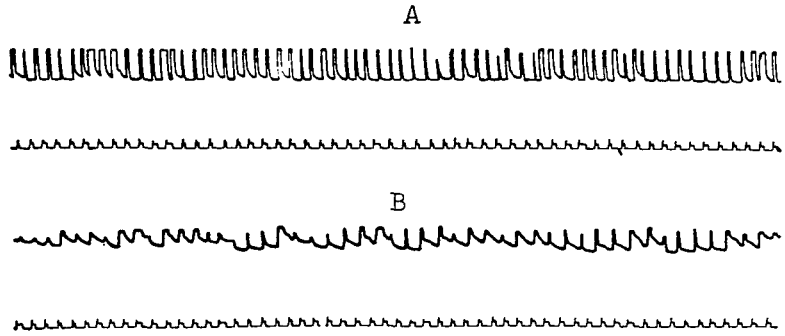

Figure 2. Recordings of breathing rate in the capucin "Caroline" at various times of the day. A, waking state (3 pm); B, during sleep (4 am). The upper curve records breathing; the lower, the time in 2-sec intervals.

with a frequency of 8-12 cps and a mean amplitude of 90-95 μV. The light stimulus in some tests caused a clear-cut inhibition of this rhythm. In the capucins, beta-like activity was more characteristic, with a frequency of 25-35 cps and a mean amplitude of 70 μV. Figure 3 shows recordings of an EKG and bioelectric activity in the brain of the capucin "Yurkiy" during a 10 cps excitation of the eyes. In most tests with the capucins, as the figure shows, the response rhythm was synchronized with the rhythm of the stimulus, as in the "secondary response" of the cortex in unanesthetized animals.

The nature of bioelectric brain activity of all monkeys during their entire stay in the restraint did not change in essentials, and no symptoms of pathological activity were observed.

The pulse rate and respiratory rhythm of capucins and macaques has been thoroughly investigated in the literature. It is known that the normal pulse rate of the macaque is 120-140 and of the capucin 180-220 beat/min. The /314 respiration rate changes from 36-38 to 40-42 per min.

Our data present evidence that under conditions of partial restraint the respiration, heart beat, and bioelectric brain activity of monkeys remain within normal limits throughout the period of partial restraint of motor activity.

If we compare our data against those in the literature, it becomes evident that chronic restraint has certain advantages over short-term restraint. Probably in the American tests their very setting (the type of capsule, constraining attachments, the experimenters, etc.) caused, in some of the animals, most severely pronounced negative reactions with each repetition, with the eventual result that they had to be rejected. Obviously, for these reasons Startsev, Utkin, and Avdzhian refer to the fact that to obtain stable indices for blood pressure,

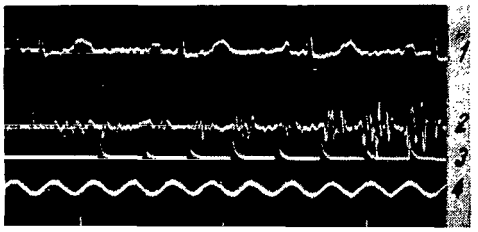

Figure 3. EKG and EEG in the capucin "Yurkiy" during rhythmic light stimulation of the eyes at 10 cps. 1, EKG; 2, EEG; 3, rhythm of stimulus; 4, time notation (0.1 sec).

respiration, pulse and other reactions in monkeys a large number of tests must be run.

If the monkey remains in a state of restraint for a long time (several days or more), acclimatization of the animal to a life of restricted movement occurs rapidly, since those factors are excluded which provoke constant excitement in the monkey in the course of getting ready for the experiment. In this manner we explain the stability of the basic physiological processes in our monkeys, which began with the very first hours of restraint.

The results of the present investigation give reason to believe that an animal under conditions of prolonged restraint of motor activity can be used as a completely reliable object of study for various problems of space biology and physiology.

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EFFECTS OF PROLONGED OPTOKINETIC STIMULATION ON THE ORGANISM

V. P. Neverov

Observation of objects alternating rhythmically in the field of /316
vision evokes in men and animals a fluttering movement of the eyes. This phenomenon, called by Barany "railroad nystagmus" (ref. 9), has been designated optokinetic nystagmus (OKN). As later studies have shown, optokinetic stimuli can cause dizziness, nausea, vomiting and similar symptoms characteristic of rocking motion (refs. 7, 8, 13 and 16).

The flight of G. S. Titov aboard "Vostok-2" proved that the symptoms of vestibular stimulation are intensified while looking through the porthole used for observing Earth's surface and clouds. These phenomena became less pronounced when the cosmonaut closed his eyes or otherwise excluded the optokinetic stimuli (ref. 2). Clinical data on vestibular disturbances also furnished considerable material indicating the significance of optokinetic stimulation in magnifying or inducing undesirable vestibular symptoms. OKN of the eyes serves as the chief mechanism for studying optokinetic reactions. OKN consists of a slow phase in the direction in which the objects are moving and a rapid phase in the opposite direction. It has been shown that after cessation of optokinetic stimulation, OKN may continue for several seconds in the same direction. This phenomenon has received the designation optokinetic postnystagmus (OKPN) and has been noted in men (ref. 12), doves, cats, dogs and monkeys (refs. 14 and 18). In studying OKN during prolonged optokinetic stimulation we discovered a new phenomenon--reverse postoptokinetic nystagmus (RPN).

Our investigation was conducted on five rabbits. The optokinetic stimulus was a rotating cylinder 2 m in diameter with 22 black stripes, each 5 cm /317
wide, on its inner surface. Rotation speed was 6 rpm. The test animal was fixed in a special holder placed on a small table in the center of the cylinder.

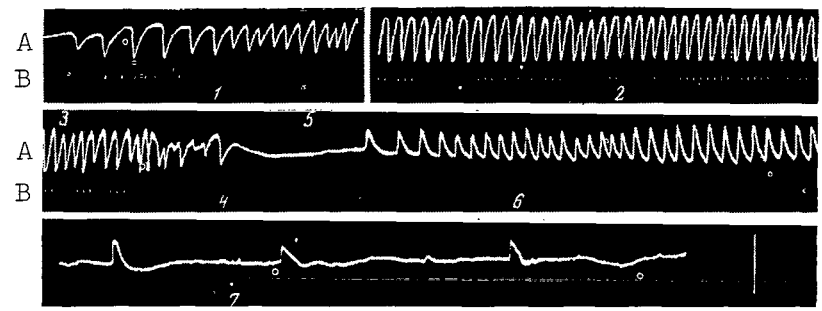
Eye movement was recorded by the electrooculographic method, which is based on recording changes in the corneoretinal potential (CRP) while the eyeball is in motion. The CRP was recorded by live electrodes at the level of external palpebral angles and was amplified by an ac amplifier. A loop oscillograph made the recordings. The duration of optokinetic stimulation was 90 min. OKN amplitude and frequency characteristics were compared after 60 and 90 min. After 90 min, cylinder rotation ceased and a black screen was placed before the animal, preventing him from seeing the stripes. After a pause, RPN developed in the fast phase, opposite to the direction of OKN. The data /318
were treated statistically by the Fischer-Student method with a confidence level $p < 0.05$.

All rabbits exhibited differences in OKN amplitude and frequency. At a rate of 6 rpm, 132 stripes per minute passed through the field of vision. However, OKN frequency was lower than this, ranging from 67.0 ± 2.9 in rabbit No. 40 to 111.0 ± 11.9 in rabbit No. 8 (table 1). Nonetheless, judging from the nystagmograms, the OKN rhythm slowed not because of asynchrony of single nystagmic movements to individual stripes, but rather because of nystagmic reaction by single movements to groups of stripes. During continuous optokinetic stimulation, a statistically significant increase in amplitude ($p < 0.05$) after 60 and 90 min was discovered only in rabbit No. 8. OKN frequency somewhat declined, with statistically significant differences found in two rabbits after 60 min and in three after 90 min.

If after a 90-min optokinetic stimulation the room was darkened or a screen was placed in front of the rabbits, eliminating the possibility of visual fixation on the stationary stripes, all of them developed RPN, whose fast /319 phase occurred in the opposite direction from that of OKN (fig.). Simultaneously with RPN, there occurred cephalic nystagmus and inclination of the trunk in a direction opposite to that of cylinder rotation. The first RPN movements appeared 10-130 sec after the screen was set up. Maximum duration of RPN was 72 min, and the average was 35 to 45 min (table 2).

Analysis of the electrooculograms (EOG) showed that the maximum RPN amplitude was lower than that of the OKN observed at 90 min. RPN frequency, which also exhibited individual fluctuations, attained OKN values and sometimes surpassed them (table 1). Table 3 shows the dynamics of RPN growth and decay with time.

In order to determine how long traces of optokinetic stimulation remained in the central nervous system, we conducted the following tests. After an uninterrupted 90-min rotation, we stopped the cylinder and omitted the screen, allowing the rabbit to see the stationary black stripes. Under these conditions RPN did not develop. After 30 min we placed the screen in front of the rabbits. All animals in this series developed RPN with accompanying cephalic nystagmus and inclination of the trunk.



Development of RPN after cessation of optokinetic stimulation. 1, OKN at beginning of cylinder rotation; 2, OKN after 60 min; 3, OKN after 90 min (arrow: moment of screen placement); 4, OKPN; 5, pause; 6, RPN; F, end of RPN; A, EOG (strip recognition); B, calibration (500 μV/sec).

TABLE 1. AMPLITUDE (in μV) AND FREQUENCY OF OKN AND RPN (per min).

Rabbit No.	No. of tests	Time of optokinetic stimulation						Amplitude and frequency of max RPN	
		2 min		60 min		90 min			
		Amplitude	Frequency	Amplitude	Frequency	Amplitude	Frequency	Amplitude	Frequency
5	6	434,0±33,4	92,0±4,6	451,0±20,5	84,0±2,3	409,0±19,0	77,0±3,7*	302,0±24,9**	62,0±17,24
7	6	268,0±31,2	95,0±6,9	325,0±50,0	86,0±6,8	331,0±35,3	78,0±4,4*	281,0±22,1	94,0±12,7
8	6	216,0±25,3	111,0±11,9	298,0±12,0*	84,0±3,6*	333,0±4,0	87,0±4,3	274,0±26,6**	135,0±8,3**
40	5	375,0±50,0	67,0±2,9	421,0±28,0	63,0±3,6	437,0±28,6*	64,0±3,6	248,0±30,5**	54,0±13,7
162	6	339,0±60,4	84,0±5,2	463,0±35,9	69,0±4,4*	472,0±74,6	63,0±4,1*	348,0±40,3**	90,0±14,7

*Statistically significant difference in OKN frequency and amplitude compared with 2 min.

**Statistically significant difference in RPN frequency and amplitude compared with 90 min OKN.

TABLE 2

Rabbit No.	5	7	8	44	162
Duration of RPN (min)	45.8 ± 6.3	43.1 ± 5.2	46.4 ± 2.3	35.5 ± 3.9	37.0 ± 3.6
No. of tests	6	6	6	5	6

TABLE 3. CHANGE IN RPN AMPLITUDE AND FREQUENCY. TEST OF NOV. 1, 1962, RABBIT NO. 162.

Time, min	1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
RPN amplitude	315	310	290	195	223	291	237	170	303	322	311	298	306	310	272	253	0
RPN frequency	96	126	120	130	126	114	96	96	78	74	72	48	54	54	36	30	0

TABLE 4. RPN AMPLITUDE AND FREQUENCY WITHOUT (I) AND WITH (II) DELAY.

Rabbit No.	5	7	8	44	162
No. of tests	10	11	12	10	12
A. Amplitude, min					
I	302.0 ± 24.9	281.0 ± 22.1	274.0 ± 26.6	248.0 ± 30.5	348.0 ± 40.3
II	162.0 ± 4.4*	205.4 ± 33.1	255.5 ± 19.6	217.0 ± 32.2	240.0 ± 41.6
B. Frequency, min					
I	62.0 ± 17.24	94.0 ± 12.7	135.0 ± 8.3	54.0 ±13.75	90.0 ± 14.7
II	54.0 ± 3.5	37.7 ±11.6*	68.0 ± 8.3*	19.2 ± 9.0*	32.4 ± 4.1*
C. Duration, min					
I	45.8 ± 6.32	43.1 ±5.27	46.4 ± 2.39*	35.5 ± 3.98	37.0 ± 3.64
II	18.5 ± 6.5*	19.2 ±3.5*	30.0 ± 4.5*	11.4 ± 5.3*	13.0 ± 4.5*

*Statistically significant difference in indices.

In analyzing the EOG it was established that the amplitude of delayed RPN did not differ statistically from the amplitude of RPN arising without delay, except for rabbit No. 5 (table 4A). The frequency and duration /320 (table 4B and C) of delayed RPN were less than those of nondelayed RPN. In a series of tests with a 60-min delay, three of the five rabbits developed RPN for 3-5 min. These tests showed that after prolonged optokinetic stimulation, the central nervous system, in the presence of stationary objects, conserves for a considerable time traces of the stimulus, and that under certain conditions these traces appear in the form of delayed RPN.

The nature of optokinetic and vestibular nystagmus compels us to suppose the presence of common nervous mechanisms for both processes, notwithstanding

their different receptors. References 4 and 11 discuss the question of the origin of vestibular nystagmus. The findings of recent neurophysiological and neuromorphological studies of Szentagothai (refs. 19 and 20), who discovered the connection of oculomotor cortical centers and the retina with the reticular formation of the corpus striatum, of Lachmann et al. (ref. 15), who discovered the mesencephalic nystagmogenic zone, and of other investigators (ref. 10), who found in the reticular formation neurons which are activated synchronously with the phases of nystagmus, lead us to suggest that precisely these structures are common to OKN and to vestibular nystagmus. The RPN which we have described, whose characteristic feature is its counter-OKN directionality, is evidently tied in with the prolonged circulation of a stimulus in the nerve structures, just enumerated, in the absence of an exogenic optokinetic stimulus. The presence of delayed RPN testifies to the prolonged conser- /321 vation in these structures of stimulus traces without transference to the motor neurons of the eye muscles.

These RPN properties permit us to regard this phenomenon as one which affords the possibility of studying neurophysiological mechanisms of illusory reactions occurring in aviation and spaceflight (refs. 1, 2 and 5). This conclusion is supported by the fact that studies with human subjects have described reverse optokinetic illusions (ref. 17).

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THE EFFECTS OF TRANSVERSE ACCELERATIONS ON THE ORGANISM OF FEMALE MONKEYS

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Training women for spaceflight has necessitated supplementary experiments on various flight factors, among them accelerations. /322

We know that accelerations can create various disturbances in the organism, among the more important of which are circulatory and respiratory disorders. We also have evidence of hemorrhaging in various organs and tissues (refs. 3-5 and 7), which under certain conditions can adversely affect the viability of the organism.

The attitude which the body of the pilot assumes in the present-day spacecraft cabin subjects him to transverse accelerations not only in the chest-to-back direction, but also in the head-to-pelvis direction. This creates conditions leading to large accumulations of blood in the vessels of organs in the lesser pelvis. Considering the anatomical and physiological peculiarities of the female organism, we naturally anticipate that this last circumstance will have adverse consequences. No experimental investigation of this problem has been made.

The literature has only a few data on accelerations experienced by women in fighter planes (ref. 6).

The authors have noted disturbances of the menstrual cycle to the extent of total amenorrhea. In the opinion of these authors, women should not fly during menstruation.

Isolated experiments on small laboratory animals have provided evidence of functional and morphological changes in ova after a single exposure to acceleration (ref. 2). /323

Monkeys were chosen as test subjects in the present work because they are the only members of the animal kingdom whose sexual cycles are similar to those of man in their morphophysiological characteristics.

The basic aim of these experiments was to study the effects of accelerations on the animal organism--and primarily on the anatomicophysiological indices of the state of the genital apparatus, the cardiovascular system, and the respiratory organs--as well as determination of the most dangerous periods of the sexual cycle from the standpoint of toleration of spaceflight conditions.

The present investigation was conducted on 16 pubescent female monkeys aged 5-8 years (5 hamadrill baboons and 11 rhesus monkeys). For 20-25 days before rotation tests all animals underwent physiological examinations at the clinic and were certified to be in good health. The phases of their sexual cycle were determined during this period by microscopic examination of vaginal smears and by changes in genital epithelium. Accelerations were applied during the following phases of the ovarian-menstrual cycle: proliferative, secretory, menstruation, and ovulation. Fourteen monkeys were sacrificed after rotation for pathomorphological studies and two were kept for subsequent observation. Four intact monkeys served as controls, one being sacrificed at each of the four phases of the cycle.

A centrifuge with a radius of 7.25 m was used to create chest-to-back accelerations building at a rate of 0.1 unit/sec. A semirecumbent posture in the chair was used (fig. 1). A special suit was employed for fixation. The animals were unanesthetized in all tests. An acceleration of 12 units was used; exposure duration varied from test to test. The criteria utilized to determine the limits of acceleration tolerance were symptoms of significant impairment of the external respiratory and circulatory function (prolonged holding of breath or cessation of breathing, noticeable bradycardia, or functional disorder of excitation, conduction and automatism of the cardiac muscle).

The appearance and behavior of the monkeys during rotation was monitored by television. Motion pictures with a sound track from the centrifuge cabin were made.

For pathomorphological studies the animals were sacrificed immediately (10-15 min) after rotation and 1, 24 and 72 hr afterward. A pathologico- /324
anatomic autopsy of the animals was performed, and the organs of the reproductive system (ovaries, oviducts, uterus and vagina) were removed for histological analysis. The organs were fixed using Carnoit's solution and Zenker formalin by Maksimov's method. Paraffin sections up to 5 μ thick were stained with azure-2-eosin, hematoxylin-eosin, and Schiff periosate by the MacManus-Hotchkiss method with a control (hyaluronidase), and also by the Hess-Hollander method.

Before being placed in the centrifuge, the monkeys were outfitted with pick-ups, dressed in special suits and fixed in the chair. Reaction to the unusual circumstances expressed itself in anxiety, efforts by the animals to free themselves, pulse acceleration, increased arterial pressure, etc. However, the animals quieted down rapidly after being placed in the centrifuge cabins, reacted satisfactorily to their surroundings and willingly ate the victuals proffered them. The physiological indices recorded testified to the absence of pathological deviations in their condition. However, all animals had an accelerated pulse. At the onset of rotation the animals gave evidence of an orientation reaction and motor disturbance. Thereafter, the animals were pressed into the chair more firmly as acceleration increased. Some of them shrieked. Up to loads of 9-10 units--and more rarely of 12 units--the animals occasionally made movements with head or trunk. At the end we were unable to find any motor /325
activity at all. The animals were practically in a state of "immobilization."

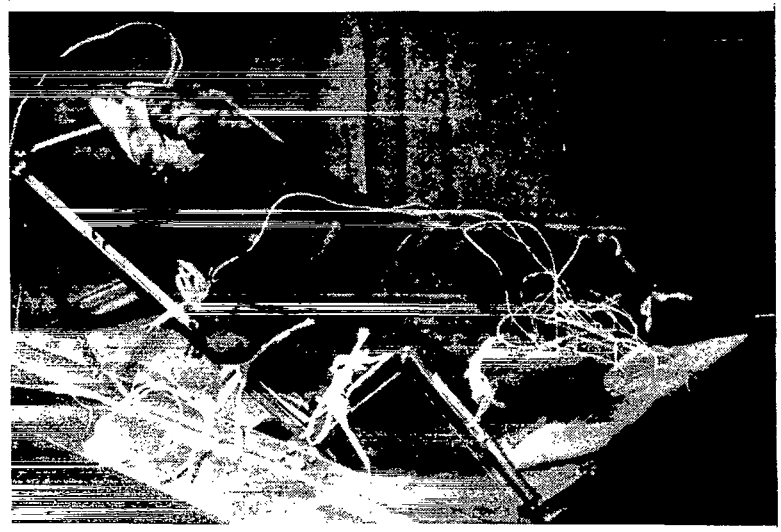

Figure 1. Position of monkey in chair.

TABLE 1. CHANGE IN FREQUENCY OF CARDIAC CONTRACTIONS AND RESPIRATION OF MONKEYS DURING 12-UNIT ACCELERATIONS (MEAN VALUES FOR 14 ANIMALS).

Frequency of	Before acceleration	During acceleration	After acceleration
Cardiac contraction	152-186	190-230	150-160
Respiration	24-36	36-48-54	18-36

Breathing increased sharply in amplitude and frequency. Visual observations revealed that in the act of inhalation the muscles of the neck and pectoral girdle played a role. At the beginning of rotation the pulse rate rose by 23.1 to 31.5 percent (table 1).

Bradycardia set in toward the end of rotation, in many cases the extent of the reduction being 60 to 80 beats/min.

EKG analysis in all cases uncovered definite sinus tachycardia with noticeable shortening of the T-R interval. The smallest changes were found in the QRS interval. Signs of impaired myocardium excitability were noted on the EKG, showing up as ventricular and strioventricular extrasystoles. In some animals extrasystole took on a polytopic character (fig. 2).

Cardiac and respiratory functions became normal in most cases 10-20 min after the centrifuge stopped.

The appearance of the animals gave evidence of their heavy physical stress: mucosa of lips, tongue, and conjunctiva were markedly cyanotic. The facial

integument was pale. Respiration remained accelerated. The monkeys were flaccid and indifferent to their surroundings. Within 5-10 minutes they resumed their customery appearance, shortness of breath disappeared, the oral cavity became pink again and they behaved in a fully active manner. In the vivarium 25-30 minutes after completion of centrifuge testing, they differed not one whit from other monkeys in their appearance and behavior: they were active and willingly ate the victuals proffered them. At the time of their subsequent sacrifice for pathologic-anatomic studies the animals gave evidence of physiological indices.

Objective evidence, as well as general observation of the behavior of /326 the animals, indicated that they were able to withstand 12-unit chest-to-back accelerations for 1-4.5 min.

Autopsies performed on 14 monkeys after acceleration revealed gross evidence of venous plethora in the organs of the lesser pelvis: ovaries, uterus, and oviducts. Plethora was especially evident in animals slain immediately after rotation, although it was also quite noticeable after 72 hours. The distribution of animals by menstrual phase and time of death after testing are presented in table 2.

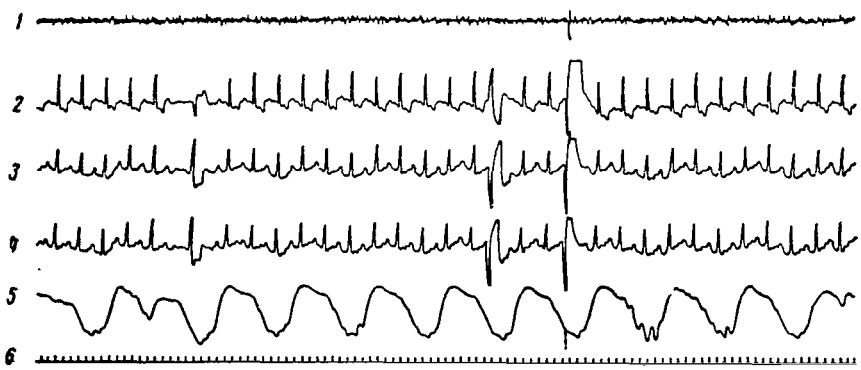

Figure 2. EKG of monkey Kamid during 12-unit acceleration. Polytopic extrasystoles. 1, EMG of thigh muscles; 2, EKG-1; 3, EKG-2; 4, EKG-3; 5, respiration rate; 6, time notation (0.1 sec).

TABLE 2. NUMBER OF MONKEYS BY TIME OF DEATH AND VARIOUS PHASES OF THE MENSTRUAL CYCLE.

Menstrual phase	Number of monkeys	Time of death after rotation			
		10 min	1 hr	24 hr	72 hr
Proliferation	3	1	-	1	1
Ovulation	6	2	-	2	2
Secretion	4	-	2	1	1
Desquamation	1	1	-	-	-

Figure 3. Monkey ovary. Proliferative phase. A, Control. Sample from cortex region. Diffuse PAS, weakly positive staining of ovarian stroma. B, Sample from cortex region 72 hours after acceleration. PAS, strongly positive staining of stroma. Schiff periodate method. Magnified 40 x 7.

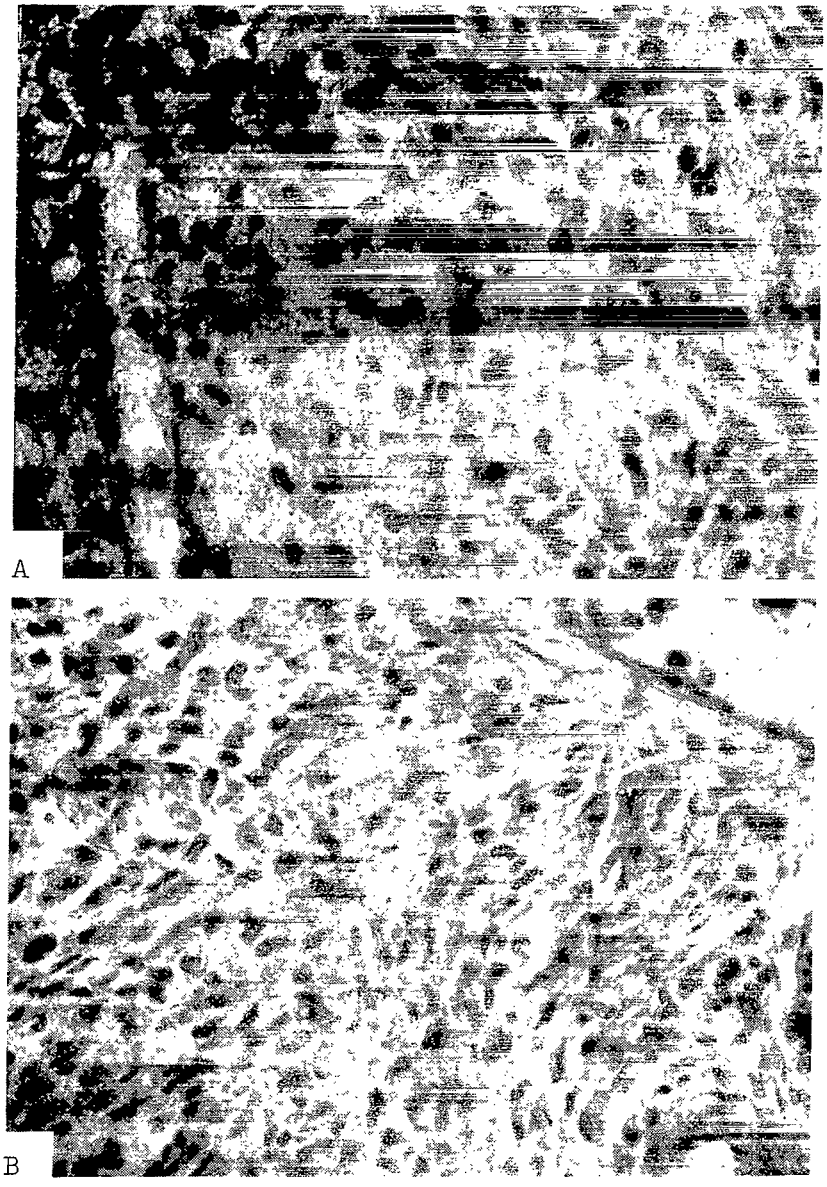

Figure 4. Ovary of monkey 72 hours after acceleration. Ovulation. Diffuse hemorrhaging in (A), medulla region and (B), cortex region. Azure-2-eosin stain. Magnified 40 x 7.

Figure 5. Monkey ovary 72 hours after acceleration. Secretive phase. Beginning of atresia of ripening follicle. Schiff periodate method, nuclei stained red with hematoxyline. Magnified 40 x 7.

Punctate hemorrhages were also discovered in the mucosa of the uterus. No such morphological changes were noted in the ovarian ducts or the vagina.

Menstruation Phase. Gross and microscopic examination established the same changes in the ovary, the ovarian ducts and the uterus, as were found in the proliferation phase.

Analysis of the cardiovascular system and respiration testify to generation of a state of extreme stress on the organism under the effects of prolonged acceleration. In fact, although at the onset of rotation a clear-cut compensatory-adaptive reaction appeared--in the form of quickened pulse and breathing /331 and the absence of pathological EKG changes. Several monkeys in the second minute and others in the third and fourth minute of 12-unit acceleration manifested definite signs of decompensation: noticeable bradycardia, extrasystoles of various origins and pronounced respiratory distress of long duration. The general state of the animals at the end of rotation and immediately afterward presented a picture of collapse.

However, these changes were transient. As a rule, normalization set in within 20-30 min after the centrifuge came to a halt. Generally the animals were exposed to 12-unit transverse accelerations for 1-4.5 min.

Naturally, in the present work, the most severe experimental conditions were created in order to obtain a clear picture of possible damage to the organs of the lesser pelvis.

The histological and histochemical analysis performed on the ovaries at various stages of the menstrual cycle showed certain deviations from the norm.

Proliferative Phase. Gross and microscopic studies of the ovaries /327 and uterus immediately after exposure to acceleration revealed no gross morphological changes. However, histochemical analysis established slight depolymerization in the acid mucopolysaccharides of the interstitial substance of connective tissue in the medulla and particularly the cortex of the ovaries (fig. 3). Depolymerization of the acid mucopolysaccharides was also found in the basement membrane of the uterine vessels. Augmented atresia of the primary and growing follicles was noted in the ovaries. No hemorrhaging was found in the uterus, ovaries and vagina. Isolated petechial hemorrhages were observed only on the third day after the end of the tests, in the ovarian cortex; they had a diapedesic character.

Ovulation. Histochemical study of ovarian preparations conducted one day and especially three days after acceleration revealed that the entire tissue of the ovary was filled with erythrocytes. In places around the wall of the venules we noted plasmorrhage and focal hemorrhage (fig. 4). One day after centrifugation, in ovary preparations stained by the Schiff periodate method, we observed a homogeneous bright-red granulation against dull-pink connective tissue stroma and around the capillaries, accompanied by disappearance of gamma metachromasia in these areas, which testifies to the abrupt depolymerization of the acid mucopolysaccharides. A similar picture appeared when the ovaries were prepared with hyaluronidase. Almost all primary and growing follicles had suffered atresia. It is of special interest that in the growing follicles, the egg cell together with a layer of follicular epithelium cells, had exfoliated from the outer covering of the follicle, the egg cell acquiring an elliptical shape in the process.

Secretory Phase. Exterior examination of two of the four monkeys revealed vaginal hemorrhage. Autopsy revealed hemorrhage in the endometrium with some bleeding in the uterine cavity. It is notable that in these monkeys onset of the desquamation phase was delayed 6-7 days. The appearance of hemorrhaging in the endometrium and the premature uterine hemorrhage must be regarded as the result of adverse acceleration effects.

Twenty-four hours after centrifugation, single acinous effusions of blood appeared in the ovaries, principally in the cortex. Erythrocytes were observed to penetrate the wall of the vessels. The vessels of the ovarian medulla showed evidence of stagnated plethora, but less pronounced than during ovulation. In the connective tissue stroma of the ovaries prepared with Schiff periodate, against a pale-pink background, we noted homogeneous pink small granular structures. Disappearance of gamma metachromasia was established on the histochemical preparations (from early periods of death up to several days). Anal- /330 ogous changes were discovered in the vessels of the uterine wall at the boundary of the submucous layer. These changes present evidence of moderate depolymerization of the acid mucopolysaccharides. We were unable to establish the presence of such abnormalities in the vagina and ovarian ducts. Most of the primary and growing follicles had suffered atresia (fig. 5). On the third day we discovered punctate hemorrhaging without plasmorrhage.

Gross and microscopic studies of the ovaries, ovarian ducts, uterus and vagina showed that the nature and degree of morphological deviation depended on the phases of the menstrual cycle.

Thus the most traumatic acceleration effects occurred in the period of ovulation, especially if we take into account the hormonal background at that period.

Acceleration during the secretive phase can also lead to uterine hemorrhages (menorrhagia) in some cases.

The most favorable period appears to be the proliferative phase. Morphological changes at this time are minimal and rare.

It is important to emphasize that as a rule the morphological changes were greatest on the third day after rotation. Histochemical analysis revealed depolymerization of the acid mucopolysaacharides in the medulla of the ovary and the walls of the vessels on the first day, which evidently indicates the gradual development of biochemical shifts, which subsequently occasioned plasmorrhages and hemorrhages. In all probability this explains the difference in severity between hemorrhages discovered immediately after the experiment and those which appeared later. So far as the pathogenic mechanisms of developing morphological changes are concerned, they await study in the future. Apparently a determining role is played by neuroendocrine effects (of the stress-reaction type) and local vessel damage. The present study is concerned essentially with most immediate consequence of accelerations. The determination of remote results is without doubt a subject of great interest. The observations made on two monkeys /332 showed that one month after centrifugation during the proliferative phase their sexual cycle was normal. One of them later had a normal pregnancy. This is possibly due to the great regenerative capacity of the ovaries following injuries of various types.

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EFFECT OF PROLONGED HYPOKINESIS ON HUMAN RESISTANCE TO ACCELERATIONS

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S. F. Simpura and I. S. Grishina

Study of the problem of hypokinesis has an important position in /333
space medicine. There is reason to suppose that prolonged weightlessness may have a deleterious effect on the ability to withstand physical stresses in conditions of increased gravitation.

It has been established that prolonged restriction of movement in a cabin of small dimensions or in bedrest lowers man's resistance to physical work (refs. 1-3, 6 and 9) and orthostatic tests (ref. 5).

Significant impairment in the regulation of blood circulation has been noted after a week's bedrest, causing weaknesses in the tonus of the skeletal musculature and even leading to orthostatic collapse (ref. 8). Persons kept in water for 5-23 hours suffered in the ability to undergo subsequent 4- and 5-unit accelerations (ref. 10). A weeklong immersion in water led to a drop in the amount of work performed by the subject in a veloergometer and in his tolerance; the pulse quickened, its pressure dropped, and a host of other impairments not present before the test made their appearance (refs. 7 and 8). The literature thus confirms that restricted movement, created experimentally in various ways, can over long periods produce essential changes in the circulatory and muscular apparatus. Similar disorders may arise in spaceflight, their intensity doubtless increasing with flight duration. In this regard the effect of prolonged hypokinesis on resistance to accelerations takes on considerable practical importance.

The purpose of the present investigation was to study the effect of /334
prolonged hypokinesis on man's resistance to transverse accelerations.

Two major stages of centrifuge testing were employed, to determine resistance in the initial state (controlled rotation) and immediately after cessation of hypokinesis. The position of the body during chest-to-back acceleration was semi-recumbent, the back of the chair describing a 25° angle from the horizontal. Accelerations were applied at a rate of 0.15-0.2 unit/sec. Each test subject was given at first a familiarization rotation of 30-40 sec at 4 units. Then two major tests were performed at overloads of 7 and 8 units, during which maximum tolerance time was determined. After hypokinesis, centrifuge rotation was conducted along the same lines. Restricted movement was ensured by rigorous bedrest conditions in the horizontal position.

The tests were conducted on five men aged 20-25 years, who had been declared fit by an expert medical team. Duration of hypokinesis was 3 days for two of the men and 20 for the other three. No physical exercise was permitted during the entire period, and the men were forbidden to lift their heads higher than pillow level or to raise themselves or to make any sharp movements of the body or extremities. During transfer to the centrifuge at the end of this phase, care was taken to preserve the subjects' horizontal position.

The condition of the cardiovascular, respiratory and other systems of the organism before, during, and after acceleration was determined from EKGs (Nebb's leads); pulse and respiration rates, pulmonary ventilation and gas metabolism (in a number of cases), the latent period of motor response to light signals and visual acuity were determined. Two-way light and sound communication with the subjects was maintained during rotation. At the end of the centrifuge phase the subjects were questioned at length about their own condition, an examination of their skin was made, and subsequently a thorough medical examination was made under clinical conditions. Peripheral blood was taken immediately before and as soon as possible after rotation for complete clinical analysis.

The basic criterion used in evaluating resistance to transverse accelerations before and after hypokinesis was the change in maximum endurance time, as well as the nature and degree of deviation from normal of fundamental physiological reactions of the organism. Here, attention was given to the subject's own /335 sensations and to observations of their external appearance and behavior.

Subjectively, the five men withstood controlled 7-unit accelerations quite well. During acceleration they experienced a feeling of considerable pressure over the entire body, especially noticeable in the chest region. Breathing was hindered. One of the men (D. A. G.) toward the end of the rotation experienced dull transient soreness in the substernal region. In two subjects there was no visual impairment. In both cases rotations were halted when the subjects gave a signal, the one when soreness appeared and the other because of fatigue. After three days of bedrest their subjective reactions to the centrifuge were the same as before. Their behavior remained quiet. There was nothing out of the ordinary in their external appearance. Time of tolerance after hypokinesis did not vary, remaining 4 min. Both subjects halted the rotation with a signal of fatigue. Table 1 presents the changes that occurred in certain physiological reactions during controlled rotation and after hypokinesis.

The figures cited in table 1 give evidence of the similarity in physiological reactions before and after hypokinesis. We were unable to find any significant differences in the level of these indices at the time of accelerations.

However, definite individual features of the reactions were found in deviations of several physiological indices. /336

As the illustrations show, the nature of changes in unconditioned breathing in both subjects during controlled rotation and hypokinesis remained substantially the same. However, in one case after bedrest oxygen deficit was much higher during the "acceleration plateau" than initially (fig. 2).

TABLE 1. CHANGES IN CERTAIN INDICES OF HUMAN PHYSIOLOGICAL REACTION DURING TRANSVERSE ACCELERATION (7 UNITS) BEFORE AND AFTER 3-DAY HYPOKINESIS (MEAN DATA)

Index of physiological functions	Test subject N. V. G.			Test subject D. A. G.		
	Initial	Before hypokinesis	After hypokinesis	Initial	Before hypokinesis	After hypokinesis
Pulse rate per min	80	132	140	89	130	141
Respirations per min	14	27	29	16	17	22
Ventilation of the lungs, l/min	7.7	13.4	14.5	6.8	13.0	17.0
Oxygen consumption, cm^3/min	330	375	500	260	360	450
Latent period of motor response/sec	0.3 0.43	0.58 0.73	0.45- 0.82	0.48 0.67	0.74	0.64- 0.76
Visual acuity	1.0	0.5	0.9	0.9	0.6	0.6

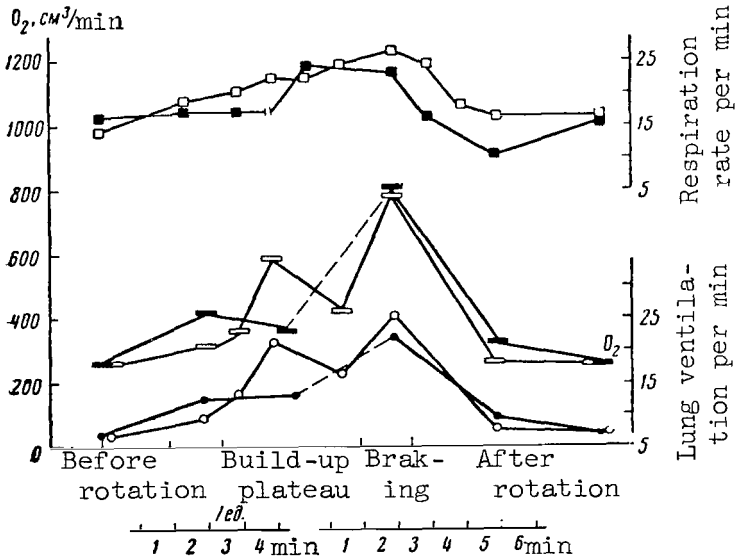

Figure 1. Change in respiration rate, ventilation of lungs, and oxygen consumption (cm^3/min) during accelerations before and after 3-day hypokinesis (subject N.). Solid line, before hypokinesis; broken line, after hypokinesis.

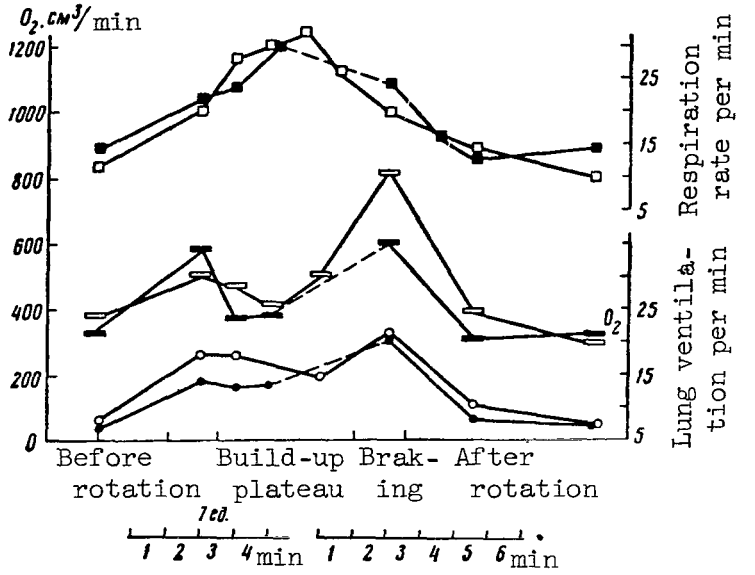

Figure 2. Change in respiration rate, ventilation of lungs, and oxygen consumption (cm^3/min) during accelerations before and after 3-day hypokinesis (subject A. G.). Solid line, before hypokinesis; broken line, after hypokinesis.

The EKGs showed sinus tachycardia without pathological deviations. The time for restoration of the indices characterizing the work of the heart and respiratory system was about the same (1-3 min).

Hemorrhages appeared in the skin of one subject, both before and after hypokinesis, and remained unchanged in character (isolated petechial spots on the back). In the other case, on the contrary, hemorrhaging after hypokinesis and rotation was observed in the skin of the posterior surface of the thorax and lumbar region, whereas after control rotation no such phenomenon appeared.

Thus, a comparison of subjective evaluations of tolerance, the length /337 of time the subjects withstood rotation and objective data show no significant differences in ability to undergo 7-unit acceleration before and after 3-day hypokinesis.

All test subjects similarly endured the 7- and 8-unit accelerations during control rotation. They noted the onset of all those sensations which are usually proper to a given amount of acceleration and the posture adopted. It became difficult to breathe. A definite muscular effort was required to take a breath. No visual impairment was observed. The subjective reactions remained satisfactory during and after rotation. After 20 days of hypokinesis the subjects were pale in the face, irascible, nervous and quite tense. As the time for rotation testing approached, agitation and a certain overalertness in their behavior became especially noticeable. After hypokinesis none of the subjects volunteered for an 8-unit acceleration, giving as their reason that they felt poorly and were experiencing fear of the rotation. However, they bore up well under 4-unit, 30-sec accelerations. No important changes in subjective /338

sensations or shifts in physiological reactions were observed compared with the control period. All bore a 7-unit acceleration with considerably more difficulty than before hypokinesis, and visual disorders occurred in the form of "grey-out" or "black-out". After rotation the subjects were even paler and manifested hyperhydrosis of the facial skin. Subjective response was still satisfactory, but there were complaints of some dizziness, which disappeared 20-40 min after the test. When emerging from the cabin under their own power they again experienced dizziness,which lasted another 5-10 min. For the next day or day and a half the subjects experienced considerable fatigue and heaviness in the head. Since they had declined to undergo 8-unit accelerations, the physiological data and time under a given stress can be compared only to the 7-unit acceleration.

Table 2 shows that the maximum tolerance time under acceleration dropped sharply in two subjects and remained the same in the third after prolonged hypokinesis. Rotation in each case was stopped at a signal from the subject. However, during rotation in the initial state a feeling of fatigue served as the signal to stop. After hypokinesis, visual disorders occurred in the form of blurring of the outlines of objects on the instrument board and either "grey-out" (in subject M-v M. A.) or complete loss of vision (in I-v L. G. and V-n V. F.). Prior to bedrest the acceleration tolerance in all subjects was approximately the same and was characterized by excellent indices in the compensatory capabilities of the organism. However, after hypokinesis /340 (also in proportion to the amount of loading) important individual differences appeared. At the time of control rotation the tolerance time for three men was approximately equal (about 5 min), while afterwards it did not change in one subject and dropped to 4 and 6 sec in the other two. A difference in the shifts of a number of physiological indices was also noted. The most notable changes were observed in the cardiovascular system.

The figures indicate that some of the subjects manifested a quickened pulse even before rotation. Subject I-v, for example, had a pulse of 78-84 during the control period and 78-84-90 after hypokinesis. During acceleration build-up this difference became more noticeable and was most clearly evident during the "plateau" phase, when it became 10-30 beats per minute. During this period respiration was also quickened.

TABLE 2. CHANGE IN TOLERANCE TIME FOR 7-UNIT ACCELERATIONS BEFORE AND AFTER 20-DAY HYPOKINESIS.

	Maximum tolerance time	
Initials of testee	Before hypokinesis	After hypokinesis
M-v M. A.	4 min 46 sec	4 min 50 sec
I-v L. G.	4 min 30 sec	4 sec
V-n V. F.	5 min	6 sec

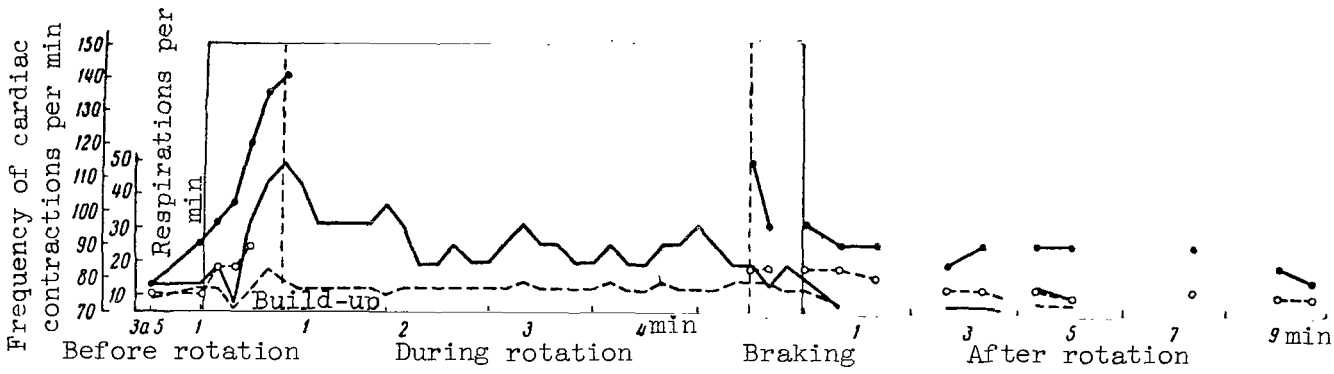

Figure 3. Change in frequency of cardiac contractions and respiration before, during and after control and postbedrest acceleration (subject I., 20-day hypokinesis). Solid line, before hypokinesis; broken line, after hypokinesis.

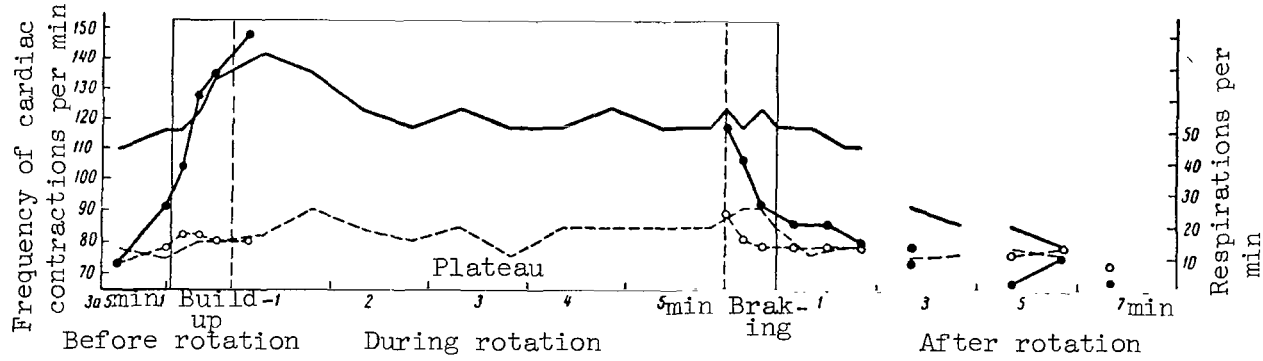

Figure 4. Change in frequency of cardiac contractions and respiration before, during and after control and postbedrest acceleration (subject V., 20-day hypokinesis). Solid line, before hypokinesis; broken line, after hypokinesis.

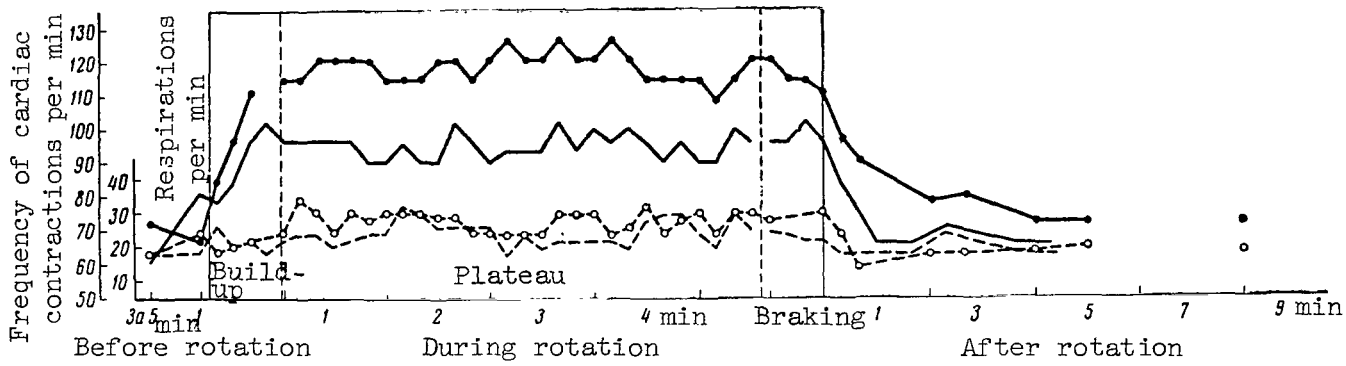

Figure 5. Change in frequency of cardiac contractions and respiration before, during and after control and postbedrest acceleration (subject M-v M. A., 20-day hypokinesis). Solid line, before hypokinesis; broken line, after hypokinesis.

The restoration time for these indices before and after hypokinesis also differed, being generally longer and more drawn out afterwards (5-10 min as against 1-3 for the control rotation). EKGs taken during the control and post-bedrest rotations showed no substantial differences, there being only a greater degree of sinus tachycardia. There were no pathological deviations in /341 the shape of the atrioventricular complex. The latent periods of motor response after hypokinesis in comparison with data in the control period did not change and remained in the usual limits (1 sec or less). Visual acuity during postbedrest rotation dropped considerably (table 3).

Analysis of the peripheral blood showed that as a result of the action of acceleration an increase occurred in the overall number of leukocytes, reaching quite significant limits 2-3 hours after cessation of rotation as a result of an augmentation in neutrophiles. Furthermore, in almost all cases during these periods after rotation, a notable reduction in the absolute eosinophil count and some drop in the absolute lymphocyte count were noted. During the same period, after cessation of rotation, there occurred in the peripheral blood a very slight reduction in the thrombocyte level. No essential changes were observed in the other indices of peripheral blood. The direction and intensity of change before and after hypokinesis was basically the same and quite specific for the effect of acceleration.

As compared with observations following control rotations, epithelial hemorrhages after postbedrest tests was noticeably greater. Notwithstanding that the total time spent on the centrifuge was greater in the control period than after hypokinesis, the number of petechiae and the general level of their appearance increased. Hemorrhaging after rotation was multiple, in places confluent, and embraced the back area, the lateral surfaces of the abdomen and chest and the loins. These petechiae persisted 2-3 days after rotation. It is appropriate to note that two of the subjects (I-v L. G. and V-n V. F.) /342 toward the end of the bedrest regime manifested changes which indicated increased tendency to brittleness of the small vessels (appearance of a positive endothelial syndrome). In one case (I-v L. G.) this tendency was accompanied by an alteration in the size and shape of the capillaries (ref. 5). These changes gave rise to considerable disturbances of the permeability of the vessel wall and to the formation of hemorrhages.

TABLE 3. CHANGE IN VISUAL ACUITY (ARBITRARY UNITS) DURING 7-UNIT ACCELERATION FOLLOWING 20-DAY HYPOKINESIS.

Subject	Initial level	Visual acuity during rotation	
		Before hypokinesis	After hypokinesis
M-v M. A.	1.0	0.7	0.4
I-v L. G.	1.0	0.8	"black-out"
V-n V. F.	0.9	0.7	"black-out"

Thus, the data of objective information, observation of the exterior appearance and behavior of the subjects and their own subjective sensations testify to reduced acceleration tolerance after 20 days of hypokinesis.

Individual differences occurred. The maximum tolerance time dropped sharply in two testees and remained unaltered in the third. However, in all cases the intensity of shifts in physiological reactions of the organism was noticeably greater. This is evidence of a greater stress on the compensatory adaptive mechanisms of the organism upon application of accelerations after prolonged hypokinesis.

Prolonged restriction of motor activity in conditions of a bedrest regime in combination with a reduction in hydrostatic pressure of the blood is the basic pathological factor causing reduced resistance of the organism to acceleration.

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EFFECT OF SMALL CORIOLIS ACCELERATIONS ON THE FUNCTIONAL STATE OF THE HUMAN HEART

R. A. Vartbaronov

A leading role in the genesis of motion sickness phenomena is /343
played by unusual and prolonged stimulation of the vestibular receptor, the source of which under flight conditions may be vertical linear accelerations in various directions (refs. 3, 10, 14 and 18), Coriolis accelerations (refs. 9, 12 and 13), angular accelerations (ref. 9), zero gravity (refs. 11 and 15), or some combination of these factors (ref. 17). In the opinion of some authors (refs. 5 and 16), the attenuated form of motion sickness must be distinguished as a separate nosological form. Investigations with slow rotation of humans in an MVK-1 (Coriolis centrifuge), with particular attention to peculiarities in the course of motion sickness brought on by prolonged action of small Coriolis accelerations, have broadened our concepts in that area (ref. 6). The basic features of this form of motion sickness consist of a long (1 to 2 hr) latent period, attenuation of clinical symptoms, prolonged (2-3 hr) aftereffect, the ability to depress certain unpleasant autonomic reactions by an act of will, and the systematic development of adaptation on cumulative exposure to Coriolis accelerations not exceeding the tolerance threshold.

The value of studying this form of motion sickness consists in the fact that similar investigations permit us, under laboratory conditions, to model certain peculiarities of prolonged spaceflight connected with the effects of rotation on the human vestibular apparatus.

In the present report an analysis is given of results of studying human cardiac activity in conditions of prolonged exposure to small Coriolis accelerations. The literature contains almost no information on this /344
problem. In reference 12, Graybiel cites T-wave inversion in one test subject during rotation for over 2 days at 5 rpm.

Two questions concerned us in these investigations: (1) to what degree does prolonged exposure to Coriolis accelerations of various intensities affect the functional state of the human heart, and (2) which indices of cardiac activity can be used as an objective criterion of vestibular-autonomic dysfunction?

The tests were conducted on four subjects with different vestibular sensitivity. An MVK-1 test stand was used with rotation speeds of 0 (control), 5.3, 10.6 and 21.2°/sec. The method of vestibular stimulation and the technical characteristics of the stand have been described elsewhere (ref. 6).

Cardiac activity was studied by recording EKGs with Mx and Dx leads (ref. 1) and seismocardiograms (SKGs) (ref. 2) on a "Kayzer" electroencephalograph. The functional capacity of the heart was evaluated from functional tests and loads: an orthoclinostatic test, V. I. Delovskaya's method for the oculocardiac reflex and a modified test with physical loading. The last consisted of raising a 7-kg weight with both arms to a height of 1 m 30 times over a period of 1 min. Quantitative data were treated statistically with computation of the mean and mean square deviations; during analysis of the results the reliability of the differences was computed.

Investigation of the functional state of the heart under control conditions revealed no abnormalities in any of the test subjects. EKG and SKG indices (pulse rate, duration of electrical and mechanical systole, magnitude of first and second T-waves) fluctuated within definite physiological limits on various days and at different times of day. One subject manifested respiratory arrhythmia (up to 0.25 sec). Monotypical changes appeared in all subjects during functional testing. During orthostatic tests we noted quickening of the pulse (by 10 to 25 beats/min) with a stabilization period of not more than 15 sec, relative and absolute increase in the first T-wave, more rarely an absolute increase in the second T-wave and an insignificant shortening of electrical and mechanical systoles correlated with the change in cardiac rhythm. During the 7-kg test similar changes were noted: the aftereffect period lasted from 20 sec to 2 min, with occasional shortening of atrioventricular conduction time.

In studying the oculocardiac reflex in the control experiments a slight depression in the pulse rate was noted in a majority of cases (2-6/min), together with a drop in T-wave voltage in both the Mx and Dx EKGs, an /346
absolute and relative reduction of the first SKG T-wave, and an unimportant increase in the duration of electrical and mechanical systole.

The cumulative (24 hr) effect of 5.3°/sec Coriolis acceleration brought about hardly noticeable fluctuations in cardiac activity, which did not differ from the values obtained in control experiments. The test with stepped Coriolis accelerations and the functional tests did not show statistically reliable departures from the control data.

In the course of the first 2-3 hr after the beginning of rotation at a speed of 10.6°/sec, three subjects gave evidence of physiological changes in the indices for cardiac activity, expressed in quickened pulse (8-10/min), shortening of electrical and mechanical systole (0.02-0.03 sec) and relative increase in the first T-wave of the SKG. A test with stepped Coriolis accelerations normalized these changes in a majority of cases. Analogous changes were noted in the fourth subject, who had excellent vestibular strength, but only at a rotation speed of 21.2°/sec. At this speed the other subjects manifested opposite reactions: a 10-16/min retardation in the rhythm of cardiac activity (a reaction which reached its nadir after 6-7 hr), lengthening of electrical and mechanical systole by 0.03-0.06 sec and an absolute and relative reduction of the first SKG T-wave. After the stepped acceleration test the pulse reaction in three subjects was greater than during control tests and at rotation speeds of 10.6 and 21.2°/sec. At the latter rate one subject, 20 sec after the test, vomited. Both before and after this reaction, considerable

tachycardia was noted, up to 110/min, with disappearance of respiratory arrhythmia. During the orthoclinostatic and physical-loading tests two subjects with high vestibular sensitivity showed significant quickening of the pulse and lengthening of the stabilization period to 40 sec, a considerable shortening of mechanical and electrical systole and a relative and absolute increase in the first SKG T-wave (statistically reliable increase over control values: see figures 1 and 2). No departures from the control data were noted in the oculocardiac reflex.

Comparison of the clinical picture of motion sickness arising in three subjects at 10.6°/sec and in all subjects at 21.2°/sec with the indices of cardiac activity permits the following conclusion.

No pathological deviations were observed in the activity of the /347
heart during the cumulative action of small Coriolis accelerations. Such changes as were noted fall within normal physiological limits. For a rotation speed of 10.6°/sec the principal effects were sympathetic in nature, while for 21.6°/sec they were largely parasympathetic, being replaced by a pronounced sympathetic effect during functional tests. In the last case, we are evidently dealing with a reduction in the functional capability of the heart as a result of the action of extracardiac factors. Attention is called to the comparatively

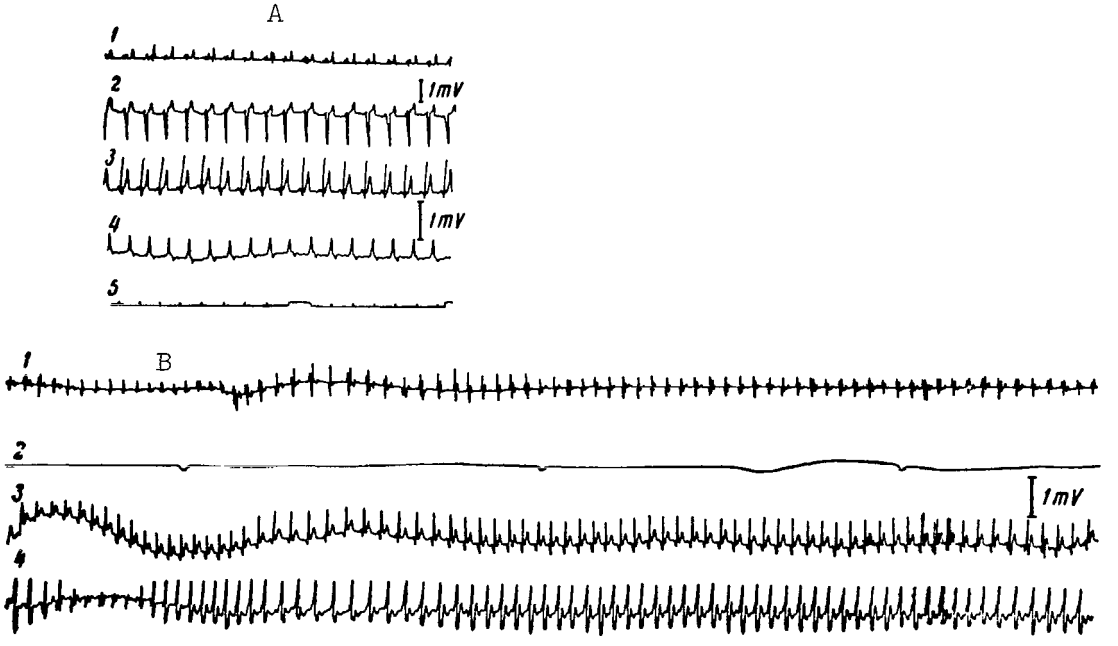

Figure 1. EKG and SKG during orthostatic tests 20 hrs after onset of rotation at 21.2°/sec (test subject V.). A, sitting position: 1, SKG; 2, EKG from Mx lead; 3, EKG from Ds lead; 4, pulse waves in phalangeal arteries; 5, time mark (1-sec intervals). B, standing position: 1, SKG; 2, revolutions of MVK-1 centrifuge; 3, EKG from Ds lead; 4, pulse waves in phalangeal arteries; 5, time mark (1-sec intervals).

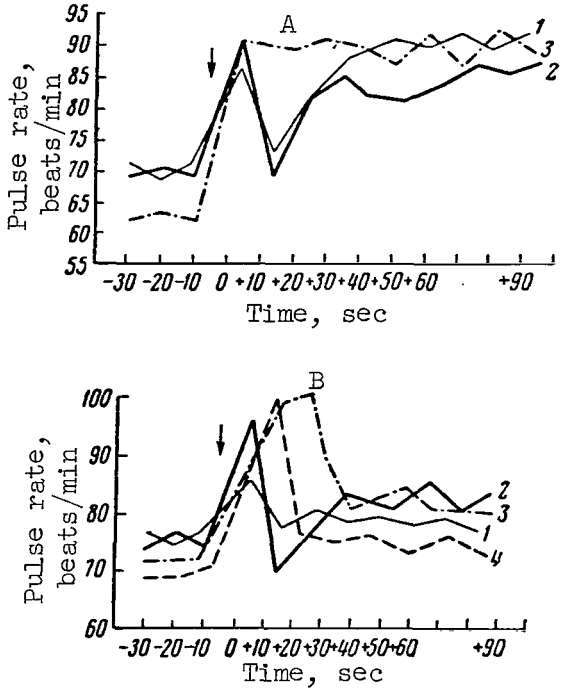

Figure 2. Change in pulse rate during orthostatic testing and prolonged rotation. A, Test subject V.; B, test subject P.; 1, 0°/sec; 2, 5.3°/sec; 3, 21.2°/sec; 4, 10.6°/sec. Arrows designate moment of transition to standing position.

small physiological deviations of cardiac activity, even in cases of clearly articulated motion sickness (judging by subjective sensations, such as chills, nausea, extreme drowsiness, apathy, etc.). We may suppose that these /348 changes are the manifestation of a general nonspecific stress reaction to the effect of an unusual rotation factor, which took form in the higher autonomic centers.

Besides this, we must consider the possibility of cardiac adaptation in the course of prolonged subjection to the cumulative effect of small Coriolis accelerations. At all events, these preliminary studies do not allow us to postulate vestibulocardiac reactions as a fine criterion of the degree of vestibular autonomic dysfunction caused by prolonged exposure to small Coriolis accelerations. However, the experimental data show high adaptability of the human heart to the action of vestibular stimuli, a conclusion which agrees with the work of other authors (refs. 7 and 8).

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CHARACTER OF EEG AND WORK CAPACITY IN MAN DURING EXPOSURE TO BACK-CHEST ACCELERATIONS

A. S. Barer and V. B. Zubavin

To evaluate the ability of the organism to withstand prolonged acceleration, as well as to study the level of compensatory reactions, many authors have investigated the changes in higher nervous activity and in the level of work capacity (refs. 3, 5, 7, 8, 10, 12 and 14-16). However, the majority of these reports deals with accelerations applied in the pelvis-head direction. /349

The present report--part of a general group of investigations into compensatory reactions of the organism in response to transverse accelerations--deals with EEG alterations in man and with the level of work capacity under back-chest accelerations.

Eighteen subjects, aged 24-34 years, participated in tests on a large-radius centrifuge.

Three series of experiments were run at angles of 65, 78 and 90° to the longitudinal axis of the body. The basic series was the one run with acceleration applied at 65°.

The accelerations increased by 2 G from one test to the next, beginning at 6 G.

The duration of the "plateau" was variable for each G load and each subject, the signal to halt the centrifuge more often than not coming from the subject himself whenever he felt from subjective sensations that he could no longer remain at the acceleration plateau.

The EEG's were recorded by two methods: with bipolar fronto-occipital leads, and with unipolar (frontal, temporal, parietal, and occipital) leads.

An Eg-138 electroencephalograph was used to make the EEG's, together with an integrator and an EA-101 analyzer. /350

One of the EEG curves was sent through the analyzer, which broke it down by frequency into the following 7 components: 1 - 2 cps (Δ_1-rhythm), 2 - 4 cps (Δ_2-rhythm), 4 - 8 cps (θ-rhythm), 8 - 13 cps (α-rhythm), 13 - 20 cps (β_1-rhythm), 20 - 30 cps (β_2-rhythm) and 30 - 60 cps (γ-rhythm).

Every 10 sec the integrator automatically calculated the integral value of each EEG component, which was the basic parameter in computing both the proportional percent of the EEG frequency components and the changes in their absolute (integral) values during the experiments (fig. 1).

At various stages in the experiment a test was made with eyes closed, during which the length of latent reaction periods was calculated, to opening and closing of eyes (refs. 1, 2 and 6), together with the level of alpha rhythm elevations, which was determined as the ratio of the integral value of a given rhythm with closed eyes to the integral value with open eyes.

The level of work capacity, studied before and after accelerations, was evaluated by the change in close motor coordination.

The apparatus for studying motor coordination consisted of an automatic programming device which fed 18 pulses, varying in intensity and direction, which deflected a pointer at 3.75 sec intervals; dials, with deflecting

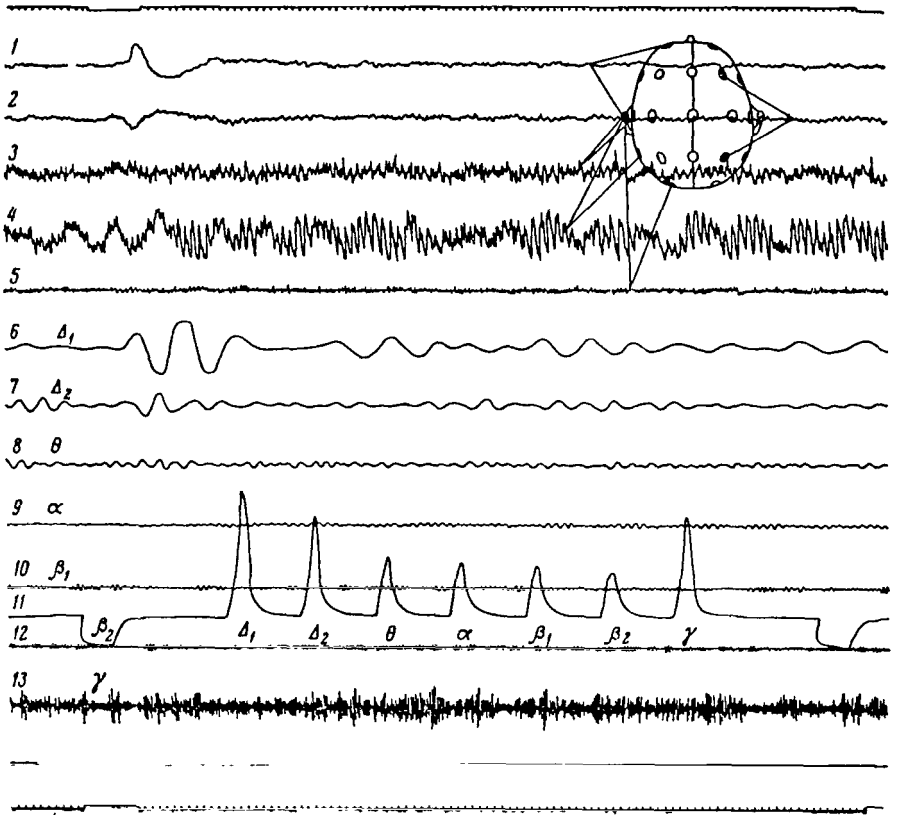

Figure 1. Example of EEG record with analysis and integration. 1, Unipolar frontal lead; 2, bipolar fronto-occipital lead; 3, unipolar temporal lead; 4, unipolar parietal lead; 5, unipolar occipital lead; 11, integration curve; 6, 10, 12, 13, analyzer channels.

pointers; control handles for resetting the pointers in the initial position (the dial and reset handle were located in the cabin with the subject), and a recording apparatus.

This device worked on the principle of requiring the subjects to reset the deflected pointer to its original position by moving the reset handle.

To evaluate the level of work capacity we calculated the latent period of motor response. This value was taken as the mean value for equal intervals of time at various stages during the period of aftereffect.

During 6 G back-chest acceleration at a 65° angle, the average endurance time for a given "stressor" was 653 sec. With this amount a certain regularity was found in the bioelectric activity of the cerebral functional system.

Attention must be given first of all to the shift toward rapid fluctuations. For example, a rhythm on the order of 20-30 cps (β_2) and 30-60 cps (γ) rose by 1.5-2 times as a percent ratio of the remaining EEG components. A /352 rhythm on the order of 13-20 cps (β_1) and 8-13 cps (α) showed individual fluctuations, most often expressed in a certain drop in its percent reading. A slow rhythm of the order of 1-2 cps (Δ_1) and 2-4 cps (Δ_2) generally also dropped for the given acceleration.

However, it is especially notable that the absolute (integral) value of all EEG components rose at the moment acceleration was applied.

At the 6 G rate the EEG reaction to opening and closing of the eyes was clearly preserved. In all cases, both before and after loading, an elevation of 1.5-2 times occurred in the alpha rhythm with the eyes closed, while plateau exaltation was higher than at the beginning of rotation (fig. 2).

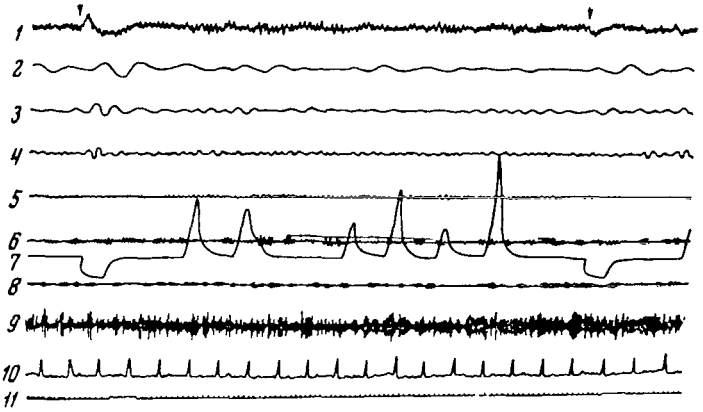

Figure 2. Character of EEG's during 6 G acceleration (arrows indicate commands to open and close eyes). 1, EEG (unipolar occipital lead); 2-6, 8, 9, analyzer channels; 7, integration curve; 10, EKG (Nebb forward lead); 11, time rotation (0.1 sec).

During latent periods of reactions to opening and closing of the eyes the following patterns were observed. The latent period of reaction to closing the eyes most frequently remained close to initial values, while the latent period of reaction to opening of the eyes decreased progressively during the experiment.

In the period of immediate aftereffect (5-15 min) all parameters being studied approximated the initial (background) level.

For an 8 G acceleration the average endurance time was 186 sec.

The dynamics of EEG change for this amount of acceleration also manifested a certain regularity.

During buildup there was a relative increase in the percent ratios of the fast components ($\beta_{1,2}$ and γ) and a decrease in those of the slow ones ($\Delta_{1,2}$). Alpha wave percent values stayed around the initial level.

However, at the very end of the plateau phase and the beginning of deceleration a characteristic shift of the "scissors" type occurred in the EEG components. The percent value of the slow waves rose somewhat, while the fast ones dropped. As in the previous test series, the absolute readings of the amplitude characteristics increased over the entire loading time (figs. 3 and 4A and B).

At the 8 G level the reaction to closing and opening the eyes underwent the same change as in the previous series, i.e., a relative increase in the level of alpha rhythm elevation on the plateau and in the period of immediate aftereffect. The latent period of reaction to closing of the eyes during overload remained close to the initial one, while the obvious phase of reaction to opening of the eyes was reduced.

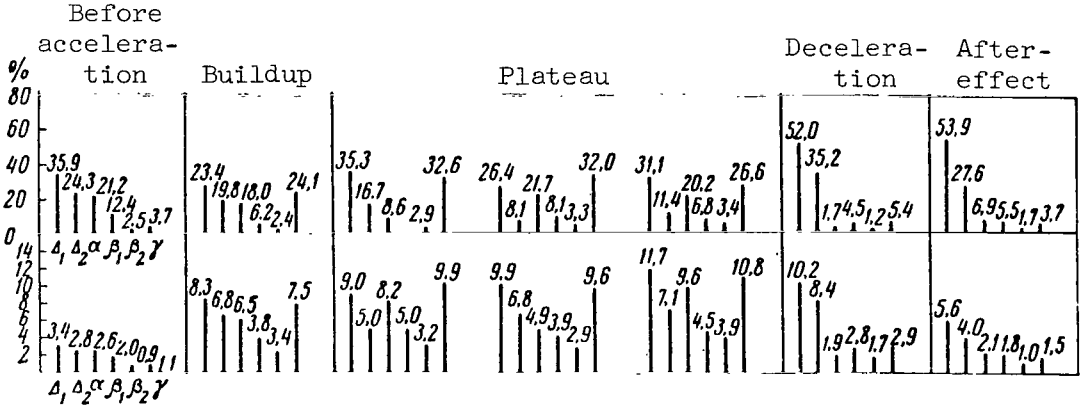

Figure 3. Change in percent content and absolute (integral) values of EEG components at acceleration of 8 G.

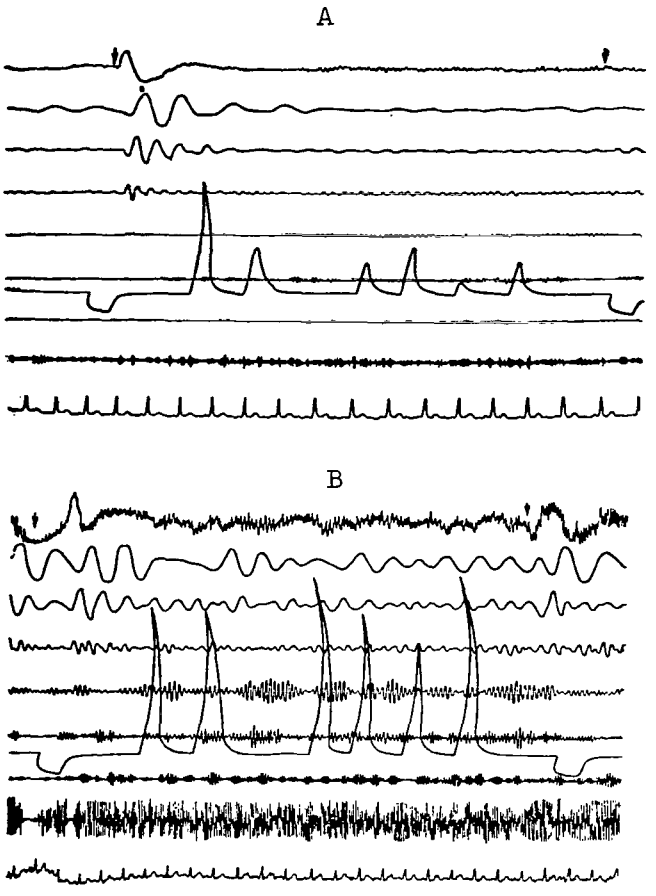

Figure 4. Change in EEG at 8 G. A, Before acceleration; B, acceleration plateau. Arrows designate commands to open and close eyes.

The endurance time for 10 G accelerations dropped to 58 sec.

The dynamics of EEG change at this acceleration showed the same /355 tendency as at 8 G, except that the scissors point described for 8 G was displaced toward the middle of the plateau phase in most cases.

The eyes-open--eyes-closed test was made only at the beginning of the 10 G acceleration and during the period of immediate aftereffect because of plateau brevity. As in the 8 G series an increase in alpha rhythm elevation was observed after loading. The definitive feature in this series was that in the period of immediate aftereffect we discovered, together with a latent period of reaction to eye closing close to the initial level, a tendency toward a certain increase in the latent period of reaction to opening of the eyes.

Special interest is presented by the loss of consciousness which one subject suffered during the 8th minute of the plateau. At the beginning of the

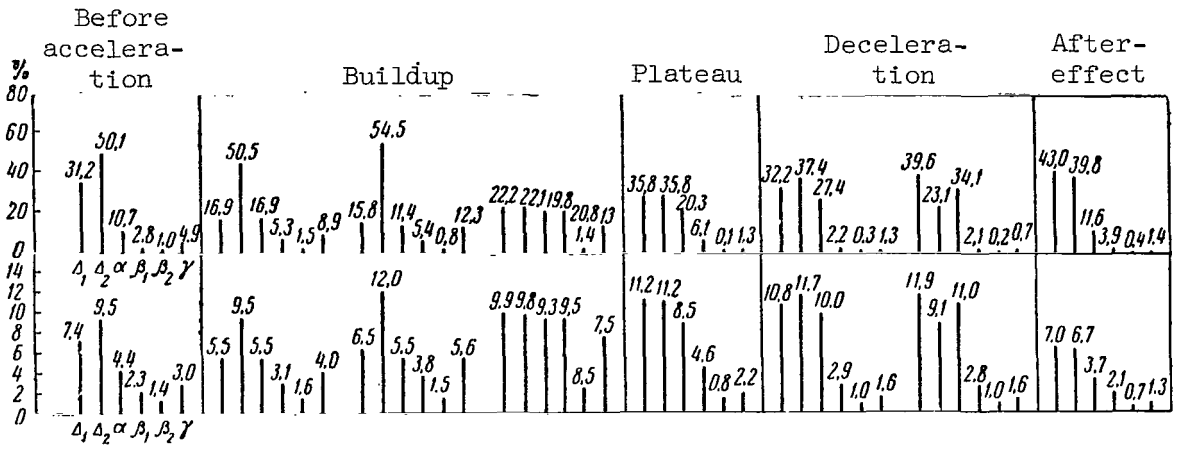

Figure 5. Change in percent content and absolute (integral) values of EEG components at acceleration of 10 G.

Figure 6. EEG at 10 G acceleration (moment of loss of consciousness).

buildup phase his EEG-change dynamics were typical for that stage (i.e., a relative increase in fast EEG components), while toward the end of the buildup period the percent and absolute values of all components began to even out, and in the plateau period, when he lost consciousness, a quite strong shift toward slow waves was noted (figs. 5 and 6).

The average time the subjects were exposed to 12 G acceleration was 28 sec.

As in the preceding series of tests, a definite regularity was noted in the changes occurring in the character of EEGs. Thus, in the first period of buildup there was a rise in the absolute values of all EEG components with a

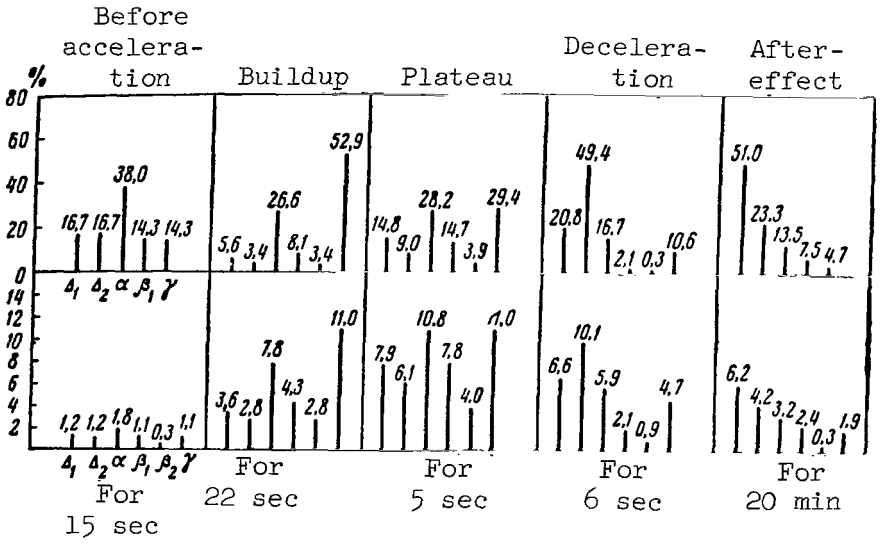

Figure 7. Change in percent content and absolute (integral values of EEG components at acceleration of 12 G.

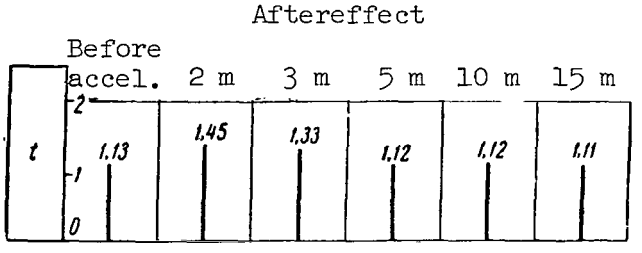

Figure 8. Latent period of motor response (in sec) before and after 14 G acceleration.

certain preponderance of fast fluctuations. However, the beginning of relative increase in slow waves, in distinction to the preceding series of experiments, was observed at the very beginning of the plateau. In the period of immediate aftereffect the EEG characteristics approximated the initial level, but in some subjects a renewed growth in the content of slow components was noted toward the 15th and 20th minute (fig. 7).

The degree of elevation of the alpha rhythm in the period of immediate aftereffect increased. The latent period of reaction to eye closing remained within the limits of the initial level, while the latent period for eye opening, as was the case in the preceding series, grew.

In experiments with accelerations of 14 and 15 G, tolerance was 18 and 10 sec, respectively.

Here the same regularities were in evidence with respect to EEG dynamics as in the 12 G tests.

For example, there was an increase in the activity of all components of the EEG during the buildup period and a selective relative rise in the slow rhythm during the period of plateau and deceleration. Afterwards an increase was /357 observed in the degree of alpha rhythm elevation and in the latent period of reaction to eye opening.

In evaluating the level of work capacity a certain relationship was noted between this parameter and the strength of the active factor. Thus, the latent period of motor response rose with an increase in acceleration. This index returned to the initial level most often in 15-20 min (fig. 8).

In line with the idea of a comparative evaluation, experiments were conducted with the effect on the subjects of 12 G accelerations in the back-chest direction at an angle of 78° to the longitudinal axis of the body.

Under these conditions the mean tolerance time was generally 2-2.5 times as great as at an angle of 65°. In our view, this is related primarily to a reduction of the acceleration component along the pelvis-head axis and cor- /358 respondingly to the lesser degree of disturbance in the supply of blood to vital organs.

During the buildup period we observed a growth in the absolute (integral) values of all EEG components with a certain preponderance of fast fluctuations. In the first period of plateau these changes continued to plunge, and the scissors phenomenon occurred only at the second half of this stage, i.e., at an unchanged level, or even with a certain drop in fast EEG components, there occurred a relative growth in slow rhythms. The period of immediate aftereffect was characterized by a return of the EEG to the initial level. In isolated cases there was afterwards some reduction in the absolute values of slow rhythms.

The level of alpha rhythm elevation underwent individual changes, most often expressed in a given value afterward remaining at a level approximating the initial one. The latent period of reaction to eye closing shortened at the same time, while practically no change was observed for eye opening.

Time endurance of 12 G accelerations at an angle of 90° was chiefly lower than at 65°, the primary factor in this difference being distress in the external respiration function (all the way up to cessation of rotation) and sensations of sickness related to displacement of the organs of the thoracic and abdominal cavities.

In this case the following regularities were observed in EEG changes.

During the buildup period there occurred a growth in absolute (integral) values of all EEG components with a certain preponderance of fast rhythms. During the plateau the EEGs remained on the same constant level, with a few individual departures, as at the end of the scattering period. During deceleration and immediate aftereffects the EEGs underwent the reverse development.

The extent of alpha rhythm elevation during the aftereffect period as a rule remained the same as prior to loading. Latent periods of reaction to eye opening and closing had individual fluctuations, but the range of change from the initial level was extremely unimportant.

Thus, evaluating the data obtained, we must first of all note that all EEG changes during 65° back-chest accelerations can be divided into two groups.

The first group, to which, in our opinion, we can relegate accelerations of 6 and 8 G, is characterized by increased activity of fast components during the plateau period, by a return of the EEG to its initial level during the period of immediate aftereffect and by insignificant shifts in the dynamics of change in latent periods of reaction to eye closing. However, even at 8 G, at the end of the plateau period, a relative increase is noted in the slow components against the background of a general rise in the level of all EEG components.

The second group contains accelerations of 12 G and above. Here the dynamics of EEG change bears an individual stamp. At the very beginning of the plateau period a selective relative increase in slow rhythms is found against a background of an increase in all EEG components. In the aftereffect period, during the 15th to 20th min, there is sometimes a rise in the content of slow EEG rhythms in comparison with the initial level. The degree of alpha rhythm elevation remained intensified for 20 min during immediate aftereffect, while the latent period of reaction to eye opening rose and had not returned to the initial level 15-20 min after rotation.

The 10 G acceleration, in our opinion, is sui generis, a transition zone between the other two.

However, in making such a cut-and-dried analysis of EEGs we must not completely exclude or fail to give attention to the fact that the biopotentials of the skeletal musculature can exert a definite influence on the frequency characteristics of the EEG potential recorded. As is known, the tonus of the skeletal musculature as a result of such effects grows to a considerable extent.

In the opinion of many authors, the appearance of slow waves in EEG's is a sign of increased activity of inhibitory processes (ref. 4).

Perhaps the reason for this is that during action of acceleration in the back-chest direction at an angle of 65° to the longitudinal axis of the body there occurs a component along the pelvis-head axis, whose value depends on the amount of acceleration. Thus, at 12 G the component along this axis is equal to 5 G. As we know, under the effect of a given component there occur definite recombination shifts, leading to hindrance of blood circulation in the brain, which, so far as we know, is the basic reason for the appearance of a slow EEG rhythm (refs. 6 and 10-13).

As proof of this proposition we cite the data of the present experiment, /360 which were obtained during the action of the human organism of 12 G accelerations at angles of 78 and 90°. In this case, in view of the smaller components along the pelvis-head axis (at 78° it was 2.5 G and was almost entirely absent at 90°), the blood supply to the brain was affected to a lesser degree. From the material

presented here it is evident that in these series of experiments even the shifts toward an increase in slow components were less intense.

Confirmation of this position was given by the work of A. S. Barer (ref. 3), who showed that the greatest degree of impairment of the conditioned reflex activity of animals arises when the direction of acceleration action conforms to that of the basic blood-carrying vessels.

We must keep in mind that activation of the inhibitory process may depend to some degree on an increased quantity of afferent impulses from the internal organs and on the immediate effect of inertial forces on the tissue of the brain.

However, during 90° tests, when the internal organs were displaced and sensations of sickness were expressed to the highest degree, no relative increase in the slow components of the EEGs were noted, as one might have supposed from the opinions of certain authors (ref. 9).

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EFFECT OF ROTATION ON THE HUMAN ORGANISM WITH THE TRUNK INCLINED AT VARIOUS ANGLES

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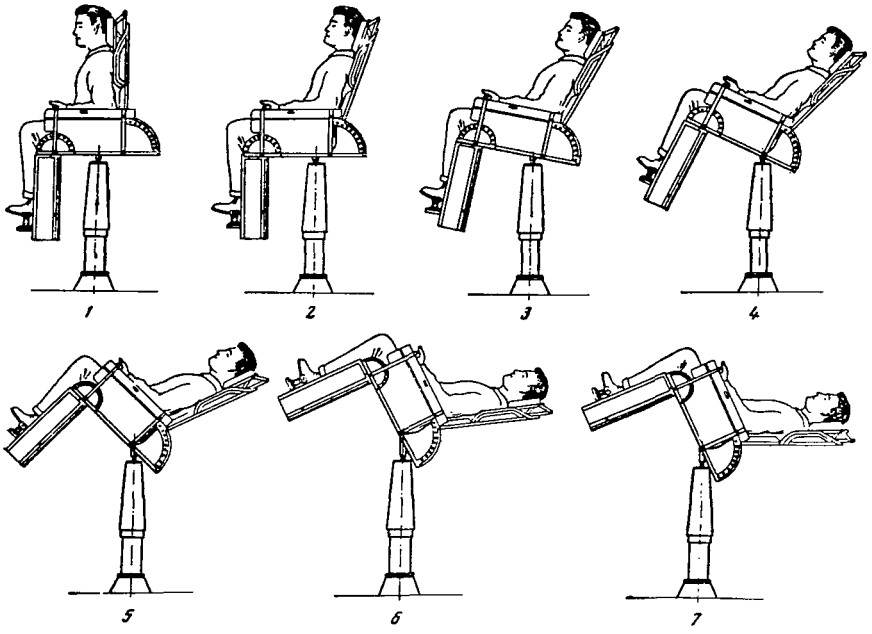
Modern flightcraft subject the human organism to various adverse /361
factors, among them lowered barometric pressure, hypoxia, acceleration, weightlessness and vibration. A number of authors (ref. 10), studying changes in the human cardiovascular system during chair rotation, have noted the appearance of bradycardia, heart blockading, missed systole, etc. These changes they connect with the reflex reaction arising in the carotid sinus region.

At the present time the study of the effect on the organism of rotation speeds and angular accelerations and the development of protective measures is one of our most pressing problems. The results of spaceflights with a human being onboard have shown that this problem is not yet fully solved, and therefore it is essential to conduct further investigation in this direction. In this connection we conducted experiments on 11 young, healthy men to determine the nature of disturbances of the functional state of the cardiovascular system and respiration. The effect of angular accelerations was studied at various rotation speeds and torso-inclination angles. One group of test subjects was exposed to angular accelerations of 30, 40, 60 and $120^{\circ}/sec^{2}$ with a constant angular rotation speeds of 1 rps. Each experiment consisted of four 5-min rotations spaced at intervals of 10-20 min. The other group was subjected to angular accelerations of 15, 30, 45, 60, 120, 180 and $240^{\circ}/sec^{2}$ at rotation speeds of 0.5, 1.0, 1.5 and 2.0 rps. Rotation duration in each case was 1.5 min, with 15-20 min between rotations. The torso-inclination angles were 0, 20, 45, 65, 80 and 90° (fig.).

Several subjects were tested 2 or 3 times. Altogether, 988 runs /362
were made. Before and after acceleration, records were made of the EKG, pulse rate and blood pressure; a chest X-ray was made as well as an X-ray motion picture of the heart and large vessels. During rotation the pulse rate, respiration and bioelectric activity of the cerebral cortex were registered. The chest X-ray was a frontal flat plate taken at the moment of maximum inhalation, while the motion picture of the heart and major vessels taken from the same angle was made at the height of a normal inhalation. The state of the organs in the thoracic cavity was studied visually and by measuring their basic parameters.



Schematic representation of body position in chair. 1, Man in chair at 0°; 2, at 20°; 3, at 30°; 4, at 45°; 5, at 65°; 6, at 80°; 7, at 90°.

Analysis of the data obtained that during angular acceleration, especially during rotation speed buildup, the subjects in a majority of cases exhibited heightened pulse rate, by an average of 10 beats/min, while respiratory frequency increased by 3. During [acceleration] plateaus at a constant speed these indices approached the initial level, at times somewhat falling below the initial data. So far as bioelectric activity of the /363
cerebral cortex is concerned, no substantial changes were noted. After the experiment all test subjects manifested a slower pulse (3 to 18 beats/min).

In most subjects systolic arterial pressure decreased by an average of 9 mm Hg, while diastolic pressure in almost all cases increased by an average of 12 mm Hg. These changes were especially clear in tests with loading.

During rotation, beginning with an inclination angle of 65°, at 80 and 90° the subjects felt a displacement of their internal organs, compression of the larynx and difficulty in swallowing. After these tests they exhibited hyperemia of the palpebral mucosa and increased blood in the vessels of the eyeball. At the moment of rotation speeds of 1.5-2 rps and a body position of 0-30°, the subjects noticed heaviness in the head and legs and inhibition of movement of the extremities.

Repeated rotations in the vertical sitting position (0°) brought about several changes in the X-ray picture of the chest organs. In most cases the

changes affected the lungs and were manifested in greater transparency of the lower lungs and in intensification of lung shadows at the radix and near-radicular areas which on later examination (5-7 days later) disappeared, leaving no visible traces on the X-rays. As is known, this intensification of the pulmonary photograph is caused chiefly by engorgement of the vessels with blood. This is related to the congestive phenomenon in the pulmonary circulatory system.

The results of X-ray studies also showed that enlargement of the shadows of the heart and major vessels, also due to an increase of blood in them, was observed in some of the subjects after testing. We also noted that in these circumstances the wave peaks on X-ray motion pictures of the heart reflecting the contractive function of the cardiac muscle underwent no substantial changes.

Test subjects exposed to repeated rotation and angular acceleration at body angles of 20-65° manifested changes connected mostly with disturbances in the circulatory system. These disturbances were quite clear both in the lungs and heart. In a majority of cases the lung picture was markedly intensified and showed a broader lung-radix shadow as a result of an augmented blood supply in the pulmonary vessels. Changes in the heart and major vessels in half the cases took the form of increased dimensions. This was occasionally accompanied by impairment of the contractive function of the cardiac muscle. Thus, for example, on X-ray motion pictures of test subject M-kov taken 20 min after cessation of the test, the following changes were noted: a sharp increase in /364
the dimensions of the heart and major vessels, and absence of pulse waves over the entire right side of the cardiac shadow and on the left ventricle (for 6 cm around the apex). In the basal area of the left ventricle the waves were split and their amplitude was less than initially. These changes on X-ray motion-picture waves over the contours of the cardiac shadow indicate significant impairment of the contractive function of the cardiac muscle. Examination of EKGs recorded at this time show that in these conditions the bioelectric activity of the heart underwent no substantial changes. The pulmonary arch also broadened, and its contours exhibited a considerable reduction in wave amplitude; on the arch of the aorta the shape of the waves did not change significantly, but their amplitude dropped somewhat. On an EKG made 24 hours after the tests were over, the dimensions of the heart were still somewhat enlarged in comparison with the initial picture; its contours conformed everywhere to the normal pattern, but the waves on the X-ray motion picture were reduced in amplitude (table).

During the succeeding clinical X-ray observations (5-7 days after testing) the changes in heart and lungs which we have mentioned disappeared entirely.

Thus, analysis of X-ray data showed that multiple angular accelerations and rotation speeds gave rise to a series of reversible functional changes in the organs of the thoracic cavity, while during a general clinical examination /365
no noticeable changes were found in the cardiovascular system.

In 50 percent of the observations, changes in the contractive function of the heart were not great and expressed themselves in a reduction of the amplitude of X-ray motion picture waves, with rounding of their peaks. In the remaining observations these changes were significant, consisting of a few cases of the

RESULTS OF LINEAR AND PLANIMETRIC MEASUREMENTS OF BASIC DIMENSIONS OF HEART AND MAJOR VESSELS ON X-RAY PICTURES (SUBJECT M-KOV).

Time at which X-ray was made	Heart			Vascular bundle	
	Transverse, cm	Length, cm	Area, cm^2	Width, cm	Length, cm
Before testing	15.2	16.7	163.0	5.9	8.6
20 min after end of testing	16.1	17.4	174.0	6.3	9.0
24 hr after end of testing	15.6	17.1	168.0	5.9	8.8
10 days after testing	15.3	16.8	164.0	6.0	8.8

absence of waves in certain sectors of the cardiac shadow, and in other cases in a change in the character of the waves that did appear.

G. F. Lang (ref.5) notes that the greatest care must be exercised in evaluating the state of the myocardium, since it is often difficult, without special examination and using only general clinical observations, to estimate the degree of change in the cardiac muscle, inasmuch as inflammation in the myocardium can be quite considerable without producing clear symptoms.

It is well known that the absence of X-ray motion picture wave on the contours of the cardiac shadow is related to changes in the myocardium (refs. 4-6). E. M. Gel'shteyn considers that in severe rheumatic myocarditis the T-wave amplitude of the left ventricle wave (myocardial insufficiency) drops beginning at the apex, the region below the apex upward along the arc of the left ventricle toward the base of the heart. M. M. Vladysik and B. M. Sosina are of the same opinion (ref. 3).

The results of our experiments have shown that some people who are less resistant to the effect of angular accelerations manifest considerable changes after rotation in the contractive function of the heart, expressed by the absence of a pulse wave in certain regions of the heart. In these cases we also find considerable enlargement of the heart, accompanied by a slowed pulse rate.

In our opinion, the appearance of a "mute zone" on the contour of the cardiac shadow after rotation is related to a reflex vasomotor disturbance of coronary blood circulation, which temporarily impairs the blood supply to the cardiac muscle. The data obtained suggest that this reflex reaction is associated with extreme stimulation of the vestibular apparatus by repeated exposure to angular accelerations.

A number of authors (refs. 2, 8 and 9) have indicated the essential role which disorders of nervous regulation in the coronary vessels play in pathogenesis of myocardial damage.

Clinical X-ray observations have established that when muscle loading is increased, its supply becomes inadequate, which gives rise to various changes. For this reason the observed changes in the myocardium occur in those areas which perform the most work. Thus the "mute zone" is often seen in the /366 region of the apex of the left ventricle.

Thus, on the basis of our material and the results of studies by other authors, we conclude that the changes observed on the X-ray heart pictures of the test subjects are evidently due to lability of their vestibular system under exposure to angular accelerations.

We consider that the results of the experimental material indicate that the observed changes in the cardiovascular and respiratory systems are functional and reversible in character.

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THE NATURE OF THE BIOLOGICAL EFFECT OF VIBRATION

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In analyzing clinical observations and experimental data regarding /367
the effects of vibration on the organism we note the polyvalency of its action. For example, during general vertical vibration of the human or animal organism there is an increase in the intensity of gas exchange (refs. 1 and 5), a shift in the activity of the cardiovascular system (rise in blood pressure, acceleration of breathing, higher frequency of cardiac contractions, etc.) (refs. 4 and 9), impairment of central nervous system activity and, primarily, disturbances in the higher nervous system (refs. 12 and 13).

An excellent bibliography of materials on the effects of vibration on the organism is given in the articles and monographs of Ye. Andreyeva-Galanina and her associates (refs. 1-3).

The larger part of the data on vibration effects relates to clinical observations and to clinical experiments. The thread of the general biological problem of the organism's interaction with the exterior medium is found in all these investigations.

Vibration as a physical factor is, of course, widely encountered in nature, and the animal world is constantly within its realm. It is natural to assume that in the evolutionary process the vibration factor had to be included in the system of interactions between the organism and its medium, along with such others as temperature, light, chemical composition, etc. Thus, many animals possess a special receptor apparatus for anesthesia of vibration energy. In fish such an apparatus is probably located in the region of the lateral line, while in insects the so-called "trichoid sensillae" serve as analogous apparatus; similar formations are discovered on the open surfaces of wings (ref. 10).

At the same time many animals, especially the more primitive forms, /368
exhibit no such apparatus. However, there are indirect indications that even these animals are capable of "sensing" vibration. We know, for example, that before a storm jellyfish move from the littoral to deeper waters; Gammarus (crustaceans), on the other hand, get as far inland at the approach of a storm as possible (ref. 11). If this phenomenon is really connected with sensitivity to remote shocks of the storm, travelling from sea to shore as low-frequency oscillations of water or atmosphere, then we must admit the possibility that any living cell (not specially adapted for that purpose) can sense vibrations. From everything said above it follows that vibration energy is apparently one of the most important ecological factors. If we further consider that with the development of technology man is subjected to increasingly intense vibration, then the problem of the biological effects of vibration takes on deep interest, from both the

theoretical and the practical viewpoint. In solving this problem the first order of business is the mechanism by which vibration acts on living systems. Concretely, we are talking here about investigations of the physicochemical, biochemical and functional properties of the cells and tissues exposed to vibration.

As we have already noted, the clinical picture of the reaction of the entire organism to vibration has been rather thoroughly studied. However, the reaction of different cells to this factor has not been sufficiently clarified. In fact, we know of only one study (ref. 9) in which it has been shown that the kidney, skin and muscles of frogs subjected to prolonged vibration exhibit increased affinity for stains. Meanwhile, study of cellular reaction should assist us to solve the problem of the specificity or nonspecificity of vibration effects.

From such reflections we came to the investigation of a host of questions related to the problem of the biological effect of vibration and decided to clarify the question of the capability of cells of higher organisms to sense the vibration energy to which an animal is subjected. Toward this end we undertook a series of experiments whose results we consider for the time being only preliminary.

The tests were conducted by the method of intravitam staining of tissues, one which permits the investigator to characterize the structural changes in cellular proteins (ref. 6). Two series of tests were made, in one of which the tissues were stained in situ, while in the other, in vitro. The experimental animals were white mice, placed in a metal cage divided into six sections, each of which contained a mouse. The cage was covered with a net and securely affixed to the vibrostand platform.

The in situ tests employed intravenous introduction of an 0.5 percent /369 solution of neutral red in a ratio of 1 ml per 50 g of live body weight. After introduction of the stain the animals underwent 30-min vibration at a constant 2 mm amplitude with frequencies from 25-75 cps.

Thus the tissues were being stained by blood circulation while the organism was undergoing vibration.

When the vibration was over, the animals were sacrificed and various organs were excised, from which the cell-bound stain was then extracted.

Further analysis and computation of the data proceeded according to a method described earlier (ref. 7). The final results were obtained as extract per gram of dry tissue weight expressed as a percent of the control (values for animals not subjected to vibration), which was taken as 100.

We studied the cerebral cortex, the cortex of the cerebellum, the subcortex (arbitrarily taken as the cerebral hemisphere with removal of the cortex), the medulla oblongata, the liver, skeletal muscle, kidney and spleen. A total of 35 tests was made. The table contains the results after statistical treatment of the data.

TABLE 1. CHANGE IN STAINABILITY OF THE TISSUES OF MICE EXPOSED TO VIBRATION (IN SITU).

Organ and tissue	Control		Frequency, 25 cps			Frequency, 50 cps			Frequency, 75 cps		
	M* ± m	%	M ± m	%	p	M ± m	%	p	M ± m	%	p
Cortex	12.6±1.1	100	14.5±0.88	115	0.211	14.3±1.15	113.5	0.287	17.6±1.06	140	0.004
Subcortex	10.3±0.9	100	22.0±3.25	212	0.001	11.5±0.76	111.5	0.331	14.0±0.73	136	0.012
Cerebellum	7.7±0.6	100	10.1±0.64	131.5	0.015	8.3±0.72	104	0.435	11.9±0.72	154	0.000
Medulla oblongata	6.2±0.6	100	7.4±0.31	119.5	0.030	5.6±0.34	90.5	0.435	9.1±0.54	147	0.005
Liver	11.6±0.7	100	11.7±0.49	101	1.000	11.9±0.71	102	0.844	13.7±0.72	118	0.051
Kidney	8.1±0.8	100	18.4±1.59	228	0.000	15.0±0.94	185	0.001	19.8±1.83	244	0.000
Spleen	11.6±1.3	100	9.3±0.27	80.1	0.128	11.2±0.74	97	0.844	10.0±0.71	86.5	0.331
Intestine	10.8±1.5	100	12.1±1.0	114	0.493	10.2±1.27	106	0.768	11.3±0.88	110.5	0.768
Muscle	1.6±0.3	100	1.9±0.05	118.5	0.435	1.6±0.85	100	1.000	2.7±0.44	169	0.042

*M--arithmetic mean in arbitrary units of extinction per gram dry tissue weight.

We determined the following consequent upon the tests.

1. The cells of an organism exposed to general vertical vibration reveal reactive changes in the capacity to sorb vital dye. Consequently, the vibration energy, directly or indirectly, reached the cells which we studied, evoking in them a nonspecific reaction.

2. The cells of various organs possess diverse sensitivity to vibration. The maximum reaction is found in the cells of the kidney, spleen and--less pronounced--in the nuclei of the subcortex and muscles. The cells of liver, intestine and particularly spleen gave weak or negative reactions.

3. The extent of reactive changes in the cells depends in a predictable manner on the vibration parameters, especially frequency. As is evident from figures 1 and 2 (sic), the reaction is more pronounced at 25 and 75 cps than at 50.

As previously noted, the experimental data are intended to clarify the question of whether all (or the larger part) of an organism's cells react to vibration when the organism is subjected to general vibration, or whether this form of energy is sensed only by special receptors.

The results of the experiments in our opinion serve to confirm the view that all cells to one degree or another are capable of sensing vibration and making an appropriate response.

The method of intravitam staining of tissues in situ, which we used to /371 conduct our experiments, makes it possible to investigate changes in the sorbtion properties of tissues throughout the organism, and equally important, at the time of vibration action.

However, a serious difficulty arises in evaluating the data obtained. The fact is that the degree of "stainability" in tissues and cells depends not only on structural changes in the protoplasmic proteins--as has been proved for cases of cell staining (ref. 6)--but also on capillary permeability and the rate of blood flow in a given organ. We might think, for example, that an increase in the stainability of kidney cells could be related to an increase in the permeability of the capillaries and with a reduction in blood flow rate. In order to decide the question of whether a change in the sorbtion characteristic of cells is related to a change in protein structure, or is caused instead by the permeability of the vessels, we conducted the second--in vitro--series of tests, where the factors of capillary permeability and blood flow rate were excluded.

White mice were again used. The animals were subjected to a 30 min vibration at a frequency of 25 cps and an amplitude of 2 mm, after which they were either sacrificed immediately or 30-60 min later. Their organs were taken out and placed in a 0.01 percent solution of neutral red for 10 min; they were then taken from the solution, and the quantity of bound stain was determined. It goes without saying that the data of this series cannot be compared with those of the first, especially in respect to absolute values, because the test conditions were different. Nevertheless, in this series also a reaction was discovered to the effects of the vibration factor, the reactive changes in the cells being preserved for some time after vibration ceased.

The data of this series of tests also confirm the fact that the cells of various tissues in the organism display varying sensitivity to the effects of vibration, although the causes of these differences are as yet unclear.

The chief conclusion we want to draw on the basis of the data obtained in the two sets of experiments is that, independently of the presence or absence in the organism of a special receptor, probably all cells sense this stimulus and react to it nonspecifically, just as to any other stimulus of sufficient intensity.

Studies of cellular reaction, in our opinion, have an important practical meaning, for they assist in explaining the primary foci of damage, which are the cause of the pathogenesis of vibration sickness. Besides, they have a theoretical interest, since they permit us to note more concrete means of investigating the mechanism by which vibration acts on living organisms. The question of the nature of the biological effects of vibration will be treated by us in further studies.

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EFFECT OF COMBINED EXPOSURE TO ACCELERATION, VIBRATION AND RADIATION ON BONE MARROW CELL NUCLEI IN MICE

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The study of spaceflight accelerations on the cells of a living /373
organism represents a complex problem. Its clarification and analysis depends on data respecting both the biological effectiveness of cosmic radiation and the interaction of radiation with other flight factors.

The effects on the mammalian cell nucleus of such dynamic flight factors as acceleration, vibration and weightlessness have been insufficiently studied to date. References 1 and 2, in which we investigated the effects of spaceflight on the cell nuclei of somatic tissues of mice, show that certain specific types of aberration occur in these nuclei: adhesion of chromosomes and occasionally a significant increase in the frequency of chromosome rearrangements.

A ground test on the effects of 70 cps vibrations over a period of 60 min, and tests directed to the effect of 35 cps vibrations on the hereditary structure of the bone marrow of mice, have shown that vibration not only affects the mitotic activity of cells, but also causes damage in their nuclei analogous to that recorded after spaceflight.

Centrifuge tests with mice in accelerations up to 8 G have shown that this physical factor, like vibration, affects the cell nucleus. The first data obtained (ref. 3), concerning the influence of vibration and acceleration on radiation effects, showed a change in the latter in the form of reduction in the frequency of chromosome arrangements and a certain increase in adhesion frequency.

The present investigation is a continuation of earlier studies and con- /374
tains comparative data of an analysis of the effect of accelerations of various duration and strength, as well as new data on the effect of radiation with combined centrifugation and vibration.

White, male mice of nonpure strains, weighing on the average 12-20 g, were used in the tests, as well as hybrid males from a cross-breeding of C57 (black) mice with white, nonpure strain mice. All tests were made with a control. After action from the test factors, the animals were killed 60 min, 4 hr and 2 days later, with subsequent fixation of the bone marrow in Carnoit's fluid. In the test on combined action of vibration, centrifugation and 350 r irradiation, the sacrifice occurred 1, 3, 7, 15 and 30 days after application of test factors. The cytological investigation was made on whole sections stained with

acetocarmine. Mitotic activity in the cells of the bone marrow was determined from a count of no fewer than 1,000 cells in various stages of division and quiescence.

The normally anticipated number of cells in the anaphase and telophase stages was compared with the number actually found in the damaged cells. Various types of chromosome rearrangements were noted: chromosome and chromatid bridges, bridges with fragments, doublet and singlet fragments and chromosome adhesion.

Investigation of the Effects of Accelerations up to 20 G

In the earlier of our two studies we investigated the effects of 5 and 15 min accelerations up to 8 G. A reliable increase in chromosome-adhesion frequency was noted. The mitotic activity of the mice's bone marrow cells remained within normal limits. Centrifugation for 5 and 15 min produced an unimportant reduction observed 4 hr later. The frequency of chromosome rearrangement after 60 min and 4 hr was higher than that of the control, principally after the longer (15 min) application of the test factor.

It was established that the increase in frequency of aberrant bone marrow cells in mice occurs as a result of increased chromosome-adhesion frequency, the frequency of that aberration depending on duration of the factor.

The present report cites data from experiments conducted for 5 min on a centrifuge with accelerations up to 20 G. The test conditions were similar to those of earlier experiments. The condition of the mice upon emerging from a 20 G test was poor. Several experimental mice could not raise themselves for 20 min or more. However, after 30 min their condition was satisfactory, and they moved normally.

Table 1 presents comparative data of cytological analysis of the effect of accelerations up to 8 G lasting 5 and 15 min and 20 G lasting 5 min. /375
A sharp drop in mitotic activity will be observed opposite the 20 G test. For all time-to-death periods the mitotic index was lower than that of the control, but especially after 4 hr (1.47 as against 2.02).

The frequency of chromosome aberration was just like that in the 8 G test: higher than the control as a result of increased chromosome-adhesion frequency. The frequency of chromosome arrangement did not exceed that observed in the control. Analysis 2 days later still showed these changes in the bone marrow. An increase was noted in the number of anaphases and telophases with large fragments. A similar phenomenon occurred in the 8 G acceleration.

These data show that the basic effect (compared with the control) of accelerations differing in magnitude (8 and 20 G) but equal in duration of exposure (5 min) is a lower mitotic activity of the bone marrow cells at 20 G, chromosome adhesion, and a certain increase in chromosome-rearrangement frequency. Data on the effect of 8 G, 15 min accelerations compared to the

TABLE 1. FREQUENCY OF NUCLEAR ABERRATIONS IN BONE MARROW CELLS OF MICE AFTER ACCELERATIONS UP TO 20 G.

Time-to-death	Action*	Cells examined		% aberrations	R	% chromosome rearrangements		% rearrangements	% adhesions	R	Mitotic index
		Total	No. of aberrations			Bridges + bridges with fragments	Fragments				
60 min	1	965	52	5,38±0,74	—	0.83±0,30	1,13±0,35	1,96±0,44	3.42±0,58	—	2,61
	2	1201	143	11,91±0,93	3,8	1,99±0,40	0.92±0,26	2,91±0.49	8,99±0,82	4,3	2,79
	3	806	95	11,79±1,13	3,6	2,23±0,51	1,36±0,41	3,59±0,66	8,19±0.96	4,0	1,54
4 hr	1	1129	121	10,71±0.92	3,1	1,95±0,41	0,44±0,20	2,39±0,45	8,32±0,32	5,3	1,98
	2	553	83	15,01±1,51	5,1	1,62±0,54	2,19±0,62	3,80±0,81	11,21±1,34	4,7	1,82
	3	1093	107	9,79±0,90	2,4	1,19±0,33	1,19±0,33	2,38±0,46	7,41±0,79	3,8	1,47
2 days	1	713	73	10,23±1,13	2,9	1,26±0,41	0,14±0.14	1,40±0,44	8,83±1,06	3,5	2,25
	2	548	48	8,76±1,21	1,7	1,10±0,45	0,73±0,10	1,82±0,57	6,93±1,08	1,9	2,17
	3	495	55	11,11±1,41	2,5	1,62±0,56	1,41±0,53	3,03±0,77	8,08±1,22	3.2	1,77
Control to 8 G test		949	59	6.21±0,78		0,84±0,30	0,95±0,31	1,79±0,42	4,42±0,66		2,48
Control to 20 G test		1198	83	6,93±0,73		1,34±0,33	1.84±0.33	3,17±0,51	3,75±0,55		2,02

*1-8 G, 5 min; 2-8 G, 15 min; 3-20 G, 5 min.

effect of 20 G, 5 min accelerations show that fundamentally both create similar changes, with a sharper drop in mitotic activity at 20 G.

Combined Action of Acceleration and Radiation

It is well known that the effect of radiation changes in relation to the medium before, during and after irradiation. Analysis of the effect of plant cell centrifugation on radiation effect (refs. 4 and 5) has shown that if centrifugation takes place before irradiation, the effect of the latter is reduced, rising when centrifugation is applied during and after irradiation. Reference 3 analyzes nuclear aberration in the cells of the spleen and bone marrow of mice subjected to combined action of vibration, acceleration and radiation. The authors showed that the radiation effect was reduced with preliminary 70 cps vibration and 8 G acceleration held for 5 min. In this work the interval between physical stressing and irradiation was 3 to 5 min and did not exceed 1 hr.

In the present report data of an analogous investigation are arrayed, but with intervals of 60 min and 4 hr between acceleration and irradiation. X-ray doses of 100 r were administered at 11 r/min. In these experiments as in those with 8 G, 5 min accelerations, the animals were dispatched 60 min, 4 hr and 2 days after centrifugation.

Table 2 presents comparative data from a cytological analysis of /377 bone marrow cells of mice subjected to combined 8 G, 15 min centrifugation and subsequent (60 min and 4 hr) irradiation. The data obtained in this experiment are compared with those obtained after X-irradiation with the same dosage.

Sixty minutes after irradiation the mitotic activity of the cells was low. A rise was noted in the following period, but it was substantially slower in the combined-action tests--not attaining the control values in two days in contrast to the test with irradiation alone.

Sixty minutes after the test with centrifugation and irradiation 1 hr later, a statistically reliable reduction was observed in the percent of nuclear damage. At 4 hr a reliable drop occurred in both combined tests, i.e., with irradiation after 1 and 4 hr. This reduction was due to a decline in the frequency of true chromosome rearrangements, both of the bridge and fragment type. This was especially noticeable 4 hr after completion of the test. During the 2 day analysis the overall percent of nuclear aberration in combined tests turned out to be equal to the frequency of aberration in the test with irradiation alone.

In the test with combined 5 min, 20 G centrifugation and subsequent 100 r irradiation after 1 and 4 hr (table 3), a sharper drop was found in mitotic activity in the first periods (60 min and 4 hr), as compared with the effect of 8 G and radiation. In the first period after death (60 min) the fusion frequency in both combined tests was higher than in the test with one dose of radiation only. The frequency of all types of chromosome aberration

TABLE 2. EFFECT OF COMBINED CENTRIFUGE ACTION WITH SUBSEQUENT IRRADIATION ON BONE MARROW CELLS OF MICE.

Time-to-death	Action*	Cells examined		% aberrations	R	% chromosome reorganizations		% rearrangements	R	% adhesions	Mitotic index
		Total	No. of aberrations			Bridges + bridges with fragments	Fragments				
60 min	1	121	62	51,24±4,54		12,40±2,98	34,71±4,35	47,11±4,54		4,13±1,81	0,53
	2	319	100	31,34±2,59	3,8	6,59±1,39	18,18±2,16	24,76±2,42	4,43	6,59±1,39	0,34
	3	471	217	46,07±2,29	1,01	7,43±1,21	31,84±2,15	39,27±2,25	1,76	6,79±1,17	0,46
4 hr	1	769	469	60,99±1,76		24,19±1,54	29,65±1,65	53,84±1,80		7,15±0,93	2,24
	2	769	350	45,51±1,79	6	12,09±1,18	20,02±1,44	32,11±1,70	3,5	13,39±1,22	1,11
	3	1314	450	34,25±1,30	5,5	10,12±0,83	18,26±1,07	28,39±1,25	11,0	5,86±0,65	1,70
2 days	1	273	23	8,42±1,68		3,66±1,12	1,10±0,63	4,76±1,28		3,66±1,12	2,16
	2	548	73	13,32±1,45		2,92±0,71	1,81±0,57	4,74±0,91		8,58±1,24	1,63
	3	1204	117	9,58±0,85		3,65±0,54	3,74±0,58	7,39±0,76		2,33±0,50	1,31
Control		1825	110	6,03±0,56		1,15±0,24	1,53±0,28	2,68±0,37		3,34±0,35	2,33

* 1-100 r; 2-8 G, 15 min, after 60 min, 100 r; 3-8 G, 15 min, after 4 hr, 100 r.

TABLE 3. EFFECT OF COMBINED CENTRIFUGE ACTION WITH SUBSEQUENT IRRADIATION ON BONE MARROW CELLS OF MICE.

Time-to-death	Action*	Cells examined		% aberrations	R	% chromosome reorganizations			R	% adhesions	Mitotic index
		Total	No. of aberrations			Bridges + bridges with fragments	Fragments	% rearrangements			
60 min	1	121	62	51,24±4,54	—	12,40±2,98	34,71±4,35	47,11±4,54	—	4,13±1,81	0,53
	2	200	103	54,00±3,52	0,4	5,50±1,60	38,00±3,43	43,50±3,50	0,6	10,50±2,16	0,25
	3	166	73	43,98±3,85	1,2	6,02±1,85	30,72±3,58	36,76±3,74	1,7	7,23±2,01	0,26
4 hr	1	769	469	60,99±1,76		24,19±1,54	29,65±1,65	53,84±1,80		7,15±0,93	2,24
	2	1561	782	50,10±1,26	5	16,72±0,94	23,07±1,07	39,78±1,24	6	10,31±0,77	1,67
	3	792	300	37,88±1,72	9	9,22±1,03	20,83±1,44	30,05±1,63	9	7,82±0,95	0,77
2 days	1	273	23	8,42±1,68		3,66±1,12	1,10±0,63	4,76±1,28		3,66±1,12	2,16
	2	629	44	6,99±1,01		1,27±0,45	3,49±0,73	4,76±0,85		2,22±0,52	1,65
	3	801	43	5,37±0,79		1,99±0,49	1,25±0,39	3,25±0,62		2,12±0,51	1,00
Control		1825	110	6,03±0,56		1,15±0,24	1,53±0,28	2,68±0,37		3,34±0,35	2,33

*1-100 r; 2-20 G, 5 min, after 60 min, 100 r; 3-20 G, 5 min, after 4 hr, 100 r.

approximated the value observed for a 100 r dosage; however, the frequency of chromosome bridges was cut in half, while fragmentation frequency was higher or almost equal to that in the test with one dose of radiation alone. In the following period (4 hr) the frequency of fusion was higher in the combined test, but the chromosome-rearrangement rate was reliably lower than in the test with a single dose of radiation.

In 2 days the frequency of all types of aberration approached that observed with the action of radiation only. However, just as with the test with 8 G acceleration, mitotic activity remained low in the combined test. When we compare the results of centrifugation with a different acceleration (up to 8 and 20 G) with subsequent radiation after 1 and 4 hr, we note that the overall percent of aberration with irradiation after 1 hr is, in the first two periods after death, considerably lower in the test with 8 G centrifugation. With irradiation after 4 hr the reduction was the same regardless of the amount/380 of acceleration. An analogous result was noted in comparing various test factors with chromosome-rearrangement frequencies (fig. 1). With respect to chromosome adhesion, its frequency was higher in combined tests with 8 and 20 G acceleration than in the radiation test, with the condition that irradiation followed centrifugation by 1 hr (fig. 3). With irradiation 4 hr after centrifugation, frequency of adhesion was higher only during the period of 60 min after death.

Investigation of Remote Consequences of the Combined Effect of Vibration, Acceleration, and a 350 r Radiation Dose

In studying the combined effect of vibration or acceleration with radiation the question arises as to the duration of the effect. Yu. S. Demin (ref. 3) has shown that after combined 70 cps vibration with subsequent 100 r irradia- /381 tion the increased frequency of mitotic damage in the bone marrow cells of mice lasted up to 10 days.

To explain the action of these factors over longer periods we conducted experiments in which irradiation either preceded or followed acceleration or vibration.

For this investigation the controls were mice subjected to only one of the three factors.

The mice were vibrated on the vibrostand under the following conditions: frequency of 700 cps, oscillation amplitude of 0.005 mm, duration of 60 min. This gave an average acceleration of 10 G.

The mice were then subjected to a 10 G acceleration for 30 min.

A 350 r dose of X-radiation was administered at 11 r/min either 24 hr before or 24 hr after vibration or acceleration.

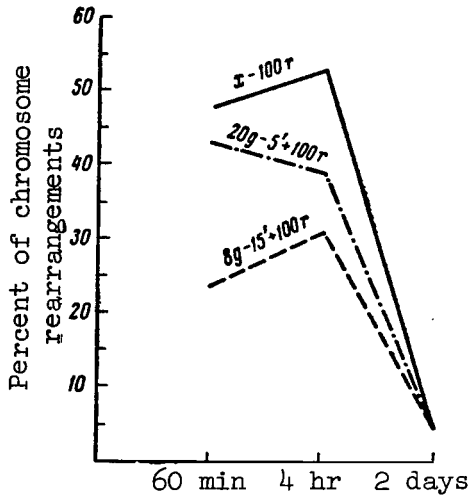

Figure 1. Percent of chromosome rearrangements following 8 and 20 G accelerations and subsequent 100 r irradiation for 1 hr.

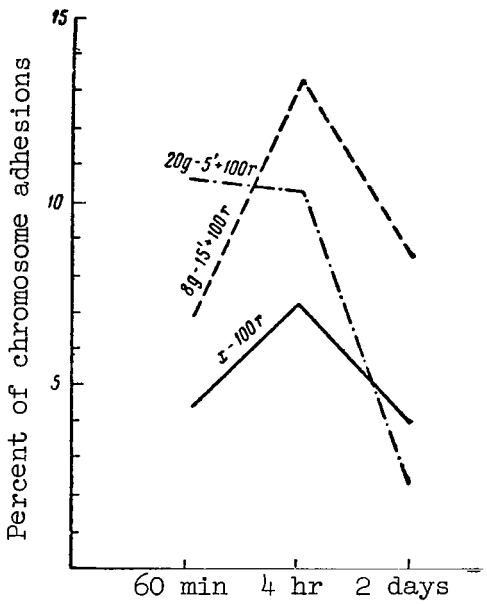

Figure 2. Percent of chromosome adhesions following 8 and 20 G accelerations and subsequent 100 r irradiation for 1 hr.

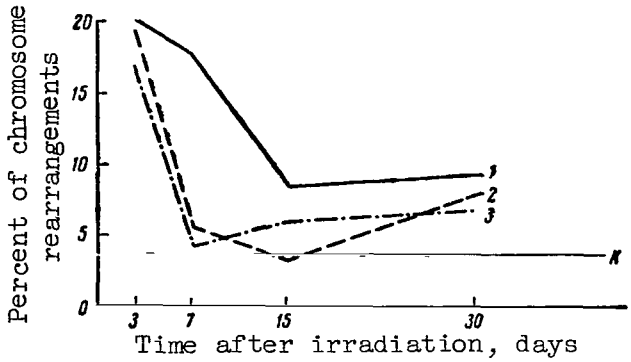

Figure 3. Frequency of nucleus aberration in bone marrow cells of mice. 1, under action of X-irradiation (350 r dose); 2, under 700 cps vibration followed by irradiation in 24 hr (350 r dose); 3, under 10 G centrifugation followed by irradiation in 24 hr (350 r dose).

In distinction to the previous test, time-to-death was 1, 3, 7, 15 and 30 days. Methods similar to those of previous investigations were used for cytological analysis of aberrations in bone marrow cell nuclei.

Outline of test No. 1: (1) 700 cps, 60 min vibration; (2) 10 G, 30 min centrifugation; (3) 350 r X-irradiation; (4) 700 cps vibration + 350 r irradiation after 24 hr; (5) 10 G centrifugation + 350 r irradiation after 24 hr.

Outline of test No. 2: (1) - (3) same as test No. 1; (4) 350 r irradiation + 700 cps vibration after 24 hr; (5) 350 r irradiation + 10 G centrifugation after 24 hr.

The biological control was carried out simultaneously. In each group and for each time-to-death period there were three gray hybrid females weighing in the average 18-20 G. In each experiment 81 mice were used (162 altogether).

Let us now examine the results obtained in the first test.

In comparison with the biological control, 700 cps vibration and acceleration up to 10 G showed for the first periods of death--viz., the 1st, /382
3rd and 7th days--a certain increase in the frequency of aberrations in bone marrow cell nuclei. A reliable difference was noted after vibration on the 1st and 3rd days. For both factors a certain increase in adhesion frequency was noted, the given effect being more clearly manifested after vibration. On the 1st to 3rd and 7th days a reliable difference was noted in comparison with the amount of adhesion in the control. The same increase was observed in the frequency of adhesion in the test with acceleration on the 30th day. The overall percent of true chromosome damage arising after centrifugation was within control limits; an increase in bridge frequency was noted on the 7th and 15th days and in fragment frequency on the 1st (table 4).

TABLE 4. FREQUENCY OF NUCLEAR ABERRATION IN BONE MARROW CELLS OF MICE AFTER 30 MIN CENTRIFUGATION AND 60 MIN VIBRATION.

Days-to-death	Load	Cells examined		% aberrations	R	% of chromosome rearrangements				% adhesions	R	Mitotic index
		Total	No. of aberrations			Br*	Br* with fragments	Fragments	% reorganizations			
1st	10 g	503	33	6.56±1.11	—	0,99	—	1.19	2.19±0.65	4,37±0.91	—	2.41
	700 cps	444	54	12,16±1,55	3,7	4,05	—	0,68	4,73±1,00	7,43±1.25	3,6	2.80
3rd	10 g	890	60	6,75±0,84	—	2,36	—	0,78	3.15±0.58	3,60±0,62	1,4	2,64
	700 cps	1020	120	11,76±1,00	4,9	4,12	0,49	1,18	5,69±0,73	5,98±0,74	3,8	2,83
7th	10 g	784	44	5,61±0,82	—	3,44	0,38	0,89	4,72±0,76	0.89±0,33	—	2,44
	700 cps	764	64	8,38±1,00	2,1	2,88	0,13	0,26	4,27±0,73	5,10±0,79	2,8	2,86
15th	10 g	769	49	6,37±0,88	—	3,77	0,26	0,39	4,42±0,74	1,95±0,50	—	2,71
	700 cps	426	26	6,10±1,16	—	1,17	—	1,88	3,05±0,83	3,05±0,83	—	2,84
30th	10 g	505	45	8,91±1,26	2,2	2,37	0,19	0,95	3,51±0,82	5,35±1,00	2,5	2,69
	700 cps	310	19	5,96±1,20	—	3,72	0,32	0,32	4,39±1,15	1,57±0,69	—	3,00
Control		1317	77	5,85±0,64	—	2,28	0,15	0,91	3,34±0,51	2,51±0,50	—	3,28

*(Br = bridges)

With respect to the effect of vibrations on the frequency of chromosome rearrangement, on the 3rd and 15th days an increase in fragments, and on the 1st, 3rd and 30th days an increase in bridges, was noted. However, all these deviations from the control were statistically unreliable.

Over the entire test period mitotic activity was somewhat lower than among the controls.

Turning to the effect of combined action of all three factors (table 5), the radiation effect merits mention.

As we know, radiation in doses of 100 and 200 r or higher sharply depresses the mitotic activity of the cell. The results of analysis for the 3rd day (material is lacking for the first day) showed that the mitotic index remained on a very low level (1.30 as against 3.28 for the control). Given such low activity and taking account also of the considerable genetic effect of such a dose (350 r), the overall frequency of damage (20 percent) discovered on the 3rd day may have been somewhat reduced, since many cells, suffering gross and vital chromosome injury, perished without attaining the division stage. Fusion percentages remained within normal limits.

With preliminary vibration or acceleration no reduction in aberration frequency was noted on the third day. A small reduction occurred in the frequency of true chromosome rearrangements as a result of reduced fragmentation frequency. Thus, the fragmentation percent for radiation only was 11.4; for acceleration with radiation, 5.3; and for vibration with radiation, 7.1. On the 7th day the overall percent of aberrations during combined action was 50 percent lower than that discovered after radiation alone. The difference is statistically reliable. The effect of such combined action was more pronounced, if we compare the frequency of true chromosome rearrangement, inasmuch as the chromosome-adhesion frequency in both cases of combined action was higher than in the test with radiation. In fact, the percent of true chromosome rearrangement is 18.12 /385 in the radiation experiment, 4.67 in the experiment with preliminary acceleration and 5.5 in the experiment with vibration + radiation (fig. 3). On the 15th day a sharp reduction in the effect was observed only in the test with preliminary 700 cps vibration, and basically because of a decrease in the frequency of fragments and bridges. On the 30th day the results of combined action and the action of radiation alone were analogous, but in all tests the number of nuclear aberrations remained well above that of the control.

This experiment confirmed data obtaining in earlier death dates with respect to reduction in radiation effect after prior centrifugation or vibration. In this test the drop was noticed on the 15th day.

In the second test of this series the effect of dynamic factors was investigated 24 hr after radiation.

A rise in mitotic activity was noticed in comparison with the preceding experiment, especially for the first period (3 days). A reduction in radiation effect was observed, but this was unreliable on the basis of comparison with the radiation effect on the 3rd day and reliable on the basis of the frequency

TABLE 5. EFFECT OF COMBINED ACTION OF CENTRIFUGATION OR VIBRATION WITH SUBSEQUENT X-RADIATION (AFTER 24 HR) ON BONE MARROW CELLS OF MICE.

Days-to-death	Action*	Cells examined		% aberrations	R	% of chromosome rearrangements				R	% adhesions	Mitotic index
		Total	No. of aberrations			Br	Br with fragments	Fragments	% reorganizations			
3rd	1	420	90	21.43±2.00	—	5.00	3.57	11.43	20.00±1.95	—	1.43±0.58	1.30
	2	75	14	18.66±4.43	—	9.31	2.66	5.31	17.29±4.37	—	1.33±1.32	0.54
	3	182	40	21.98±3.06	—	10.45	2.19	7.14	19.78±2.95	—	2.20±1.08	0.51
7th	1	629	121	19.24±1.57	—	8.90	1.43	7.79	18.12±1.51	—	1.11±0.41	2.30
	2	471	41	8.70±1.30	5.1	2.54	0.22	1.91	4.67±0.96	7	4.03±0.90	2.10
	3	291	25	8.59±1.64	4.7	4.12	—	1.37	5.50±1.33	6	3.09±1.01	0.49
15th	1	748	78	10.43±1.12	—	6.02	0.13	2.54	8.69±1.06	—	1.74±0.48	3.00
	2	390	36	8.45±1.41	1.1	3.33	0.25	2.56	6.15±1.19	1.6	3.03±0.87	2.72
	3	367	27	7.40±1.37	1.7	1.90	—	1.09	3.00±0.80	4.3	4.40±1.07	3.02
30th	1	648	68	10.03±1.15	3.1	6.64	0.29	2.36	9.29±1.11	4.9	0.74±0.33	2.29
	2	357	37	10.36±1.61	2.6	3.92	0.58	2.90	7.34±1.38	2.8	3.08±0.94	3.01
	3	383	43	11.23±1.61	3.1	4.69	0.26	2.61	7.57±1.35	2.9	3.66±0.95	3.03
Control		1317	77	5.85±0.64		2.28	0.15	0.91	3.34±0.51		2.51±0.50	3.28

*1 - 350 r; 2 - 10 G + 350 r; 3 - 700 cps + 350 r.

of true chromosome rearrangements on the 7th day. On the 15th and 30th days the effect was the same as that with radiation alone, i.e., higher than the frequency of nuclear damage among the controls (fig. 4, table 6).

Data on the analysis of chromosome aberration in bone marrow cells of mice subjected to centrifugation or vibration again confirmed that these factors evoke injuries in the cell nuclei, causing chromosome adhesion which /387 can lead to formation of pseudobridges. We must also mention the reduction in mitotic activity, which was quite severe after 20 G acceleration.

The data obtained from combined action indicate the dependence of the radiation effect on those factors to which the animals were subjected either before or after radiation. It was discovered that the radiation effect drops more sharply with preliminary centrifugation or vibration and less sharply when these follow.

In all tests with combined action the reduction in radiation effect was caused by reduction of true chromosome-rearrangement frequency, basically of fragments. However, the duration of this singular effect depended both on the time of exposure to the physical factors before irradiation and on the amount of acceleration, vibration and radiation applied.

Thus, in the test with 8 G, 15 min centrifugation followed by 100 r irradiation after 1 hr, a drop of as much as 50 percent in chromosome-rearrangement frequency was already visible 1 hr later; in the same test with radiation after 4 hr, the same reduction occurred 4 hr following irradiation.

An analogous effect with 20 G centrifugation and 100 r irradiation was noted only after 4 hr, and more severely with a 4 hr interlude between acceleration and dosing.

With more powerful radiation (350 r 24 hr after centrifugation of /388 vibration), a tangible reduction was observed on the 7th day.

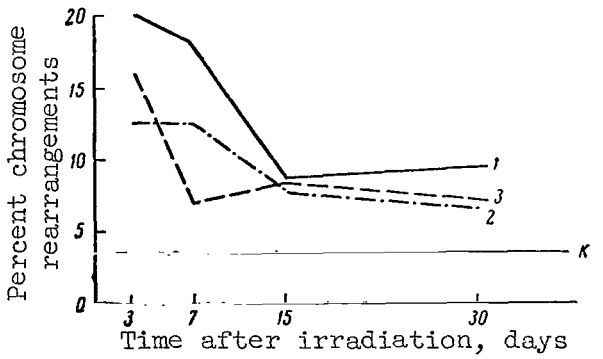

Figure 4. Frequency of nuclear aberration in bone marrow cells of mice. 1, During action of X-rays (350 r); 2, during X-irradiation (350 r) with subsequent (24 hr) 700 cps vibration; 3, during X-irradiation (350 r) with subsequent (24 hr) centrifugation up to 10 G.

TABLE 6. EFFECT OF COMBINED ACTION OF X-RADIATION AND SUBSEQUENT (24 HR LATER) CENTRIFUGATION OR VIBRATION ON BONE MARROW CELLS OF MICE.

Days-to-death	Action*	Cells examined		% aberrations	R	% of chromosome rearrangements				R	% adhesions	Mitotic index
		Total	No. of aberrations			Br	Br with fragments	Fragments	% reorganizations			
3rd	1	420	90	21,43±2,00		5,00	3,57	11,43	20,00±1,95		1,43±0,58	1,30
	2	430	80	18,60±1,88	1,0	2,53	0,46	12,88	16,08±1,76	1,4	2,56±0,76	1,90
	3	397	77	19,40±1,98	0,9	3,27	—	10,32	12,60±1,66	2,8	5,80±2,09	2,15
7th	1	629	121	19,24±1,57		8,90	1,43	7,79	12,12±1,51		1,11±0,41	2,30
	2	369	49	13,28±1,76	2,4	7,59	0,54	0,54	8,67±1,46	4,5	4,61±2,04	3,25
	3	349	49	14,04±1,86	2,1	4,81	1,65	6,59	12,61±1,78	2,9	1,43±0,63	2,77
15th	1	748	78	10,43±1,12		6,02	0,13	2,54	8,69±1,06		1,74±0,48	3,00
	2	337	37	10,98±1,70		7,54	0,58	1,45	9,79±1,62		1,19±0,59	2,88
	3	343	43	12,54±1,78		7,29	0,28	1,46	9,04±1,55		3,50±0,99	3,00
30th	1	678	68	10,03±1,15	3,1	6,64	0,29	2,36	9,29±1,11	4,9	0,74±0,33	2,29
	2	327	27	8,26±1,52	1,4	7,25	—	1,22	8,26±1,52	3,1	—	2,50
	3	339	29	8,55±1,51	1,6	6,19	0,59	0,59	7,08±1,39	2,5	1,74±0,65	2,99
Control		1317	77	5,85±0,64		2,28	0,15	0,91	3,34±0,51		2,51±0,50	3,28

*1 - 350 r; 2 - 10 G + 350 r; 3 - 700 cps + 350 r.

As we know, radiation in doses of the order of 100 r depresses and extends the cycle of the cell, while doses of 200 r and more lead to temporary arrest of cell division. We have also shown that centrifugation and vibration, especially at magnitudes like 20 G, affect the mitotic activity of bone marrow cells.

In the complex action of two factors depressing cell activity, a still longer extension of the cell cycle is possible, and the time during which combined action is manifested depends on the magnitude of the physical factors and the radiation dosage. Either the effect appears immediately after completion of the experiment or it is put off to later periods.

When radiation precedes the dynamic factor, the time between the two is of great importance. For example, in our test with 350 r, followed after 24 hr by 10 G centrifugation or 700 cps vibration, the reliable reduction in true chromosome-rearrangement frequency occurred after 7 days, while in Yu. S. Demin's work (ref. 3), where a 60 cps vibration was applied immediately after 100 r irradiation, the reduction was noted after 5 hr and 2 days.

It is possible that chromosome adhesion called into being by the dynamic factors in some measure induces healing of the ruptures created by radiation, when the latter follows acceleration or vibration, and at the same time reduces the possibility of recombination. When radiation precedes, chromosome adhesion may impede the appearance of a number of rearrangements, or again it may assist in healing potential ruptures caused by radiation. In both cases we have evidence from the reduction of both fragmentation and bridges. In fact, the percent of fragments dropped during complex action with subsequent 350 r irradiation on the 3rd day. On the 7th day a still sharper reduction was noted both in fragments and bridges. Beginning with the 15th day, the frequency of damage remained above the control, approaching in the combined test the magnitude of aberration found in the test with radiation alone.

In this manner, a considerably higher frequency of bone marrow cell aberrations was noted after one month than in normal, unirradiated controls. The overall percent of chromosome rearrangements in the control was 3.34; of this total, single fragments comprised 0.91 percent and bridges with fragments, 2.43 percent. Normally only chromatid bridges appear, and if chromosome bridges do appear they represent a negligible percent (0.15 to 0.2). A detailed /389 analysis of chromosome rearrangements in the test with 350 r irradiation showed that along with chromatid bridges and single fragments a rather high percent of chromosome bridges was noted. For the 30-day test the overall percent of rearrangement was 9.29, of which chromosome bridges comprised 3.68 percent; single fragments, 2.35 percent and chromatid bridges, 3.26 percent. Comparison of these data with values observed in the control shows that one month after irradiation the bone marrow cells retain a high percent of nuclear aberration, particularly chromosome bridges with fragments and without fragments, which testifies to the prolonged retention consequent upon radiation. Further detailed analysis of this phenomenon will permit us to define more precisely the nature of such remote consequences.

The use in these experiments of X-irradiation in doses of 100 and 350 r and also the insufficiently fractionated time-to-death make it impossible to analyze the data obtained in relation to the susceptibility of separate phases of the cell cycle. However, the appearance of fusion 2-3 days after centrifugation or vibration, as well as the reduction in radiation effect 7 days later, is evidence that the stages most sensitive to these two factors are the presynthetic and DNA synthesis. Analysis of the susceptibility of the phases of the cell cycle in the bone marrow of mice to the effects of 50 r doses has shown that the maximum susceptibility falls in the prophase state and the period of synthesis (ref. 3).

It is also known that such factors as acceleration and vibration cause in the organism of animals various impairments of their normal physiology (refs. 6 and 7).

A series of works like ours, including those of foreign authors (refs. 8-13), has shown that during action of acceleration of varying magnitude the organisms of mammals become subject to respiratory distress and impairment of blood supply. This damage leads to reduction in the vital capacity of the lungs, quickened respiration, sluggish blood flow and reduced ventilation of the lungs. The oxygen supply of the tissues and organs is also cut down.

It is possible that these changes occasion some effect on the supply of oxygen to the bone marrow of an animal. However, cell aberrations can arise not only from a change in the supply of oxygen to the tissue, all the more since the oxygen pressure in the intercellular matter of the bone marrow is quite low (10 mm Hg) in comparison with subcutaneous cell tissue (50 mm Hg).

Furthermore, we must also consider that impairment of oxygen supply in the tissues of an animal may lead to a series of reflex reactions, particularly to pronounced stimulation of the sympathetic system, with liberation of considerable quantities of adrenalin and noradrenalin. It is well known /390 that these two hormones possess strong protective action against radiation, but it has also been established that they themselves strongly repress mitosis, an effect which appears before prophase, so that the cells do not go into mitosis. The number of such cells continues to grow until finally, when the adrenalin is washed out of the tissue, all the cells split immediately (refs. 14 and 15).

During action by such physical factors as centrifugation and vibration, especially in large magnitudes, direct action on the cell apparatus can occur: chromosome displacements, detachment of chromosomes from the spindles and, finally, chromosome adhesions in various stages of the cell cycle.

In such manner the change of the normal course of cell division may occur both in the organism itself by means of hormonal and immunological action and artificially by means of a large number of various physical and chemical factors. It is evident that the basis is repression of some process preparing the division and damage to the functions of the division apparatus. Probably the duration of various actions or aberrations in cell exchange can lead to extension of the cell cycle, to repression of DNA synthesis and to the rise of chromosome aberrations.

Our discussion indicates that various physiological changes, produced by the action of acceleration or vibration, bring about a series of aberrations in cell division, which in turn may give rise to a peculiar "protective" effect against radiation.

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ADAPTATIONAL READJUSTMENTS IN THE ORGANISM OF MICE DURING AND AFTER EXPOSURE TO ELEVATED CO_2 CONCENTRATIONS

A. I. Koreshkin

One of the inauspicious phenomena which may appear in a spacecraft /391
atmosphere is increased partial pressure of CO_2. The reaction of the organism to the effects of CO_2 merits study both from the practical standpoint of determining permissible CO_2 concentration and the theoretical one of solving certain problems related to adaptive reorganization in living organisms.

Existing data indicate that the CO_2 concentration in a cabin must be maintained at 1 percent or less; higher percentages (for example, 2 percent) give rise to quite pronounced uncompensated acidosis, a reduced respiratory quotient and other departures from normal (ref. 16). Prolonged CO_2 action is accompanied by disappearance or diminution of these changes. In other words, the organism adapts to them. The possibility that animals can adapt to 10 and 20 percent CO_2 has been shown by Walker (ref. 18). In reference 2, however, I. I. Golodov tended to dispute the ability of animals to become used to high CO_2 concentrations. Against this background of controversy we decided, before everything else, to answer the question of the possibility of adapting to a given agent.

By the word "adaptation" we understand a reduction in susceptibility and an increase in the capability of a living system to resist this or that agent during prolonged action by the latter. In the first place such a definition is in harmony with the established classical concept of the adaptation of receptors (ref. 14), sense organs (ref. 7) and reflex arcs (ref. 13); in the second place, it disputes neither the concept of Selye (ref. 17) about the general adaptive syndrome nor the view of N. V. Lazarev (ref. 7) regarding /392
the state of nonspecifically increased resistance in the organism.

Both in this report and in the references the matter at hand is a general reaction (general adaptation) of the organism, the basic criterion of which is the resistance or autodefense of a living organism to the alterative effects of external factors; a supplementary criterion is the susceptibility of the organism's separate systems.

It should be emphasized that we are dealing here only with those adaptive reactions--or that partial case of adaptation--which arise as a result of prolonged action at a constant intensity. There is reason to suppose that such a limitation of the experiment permits us to follow more clearly the kinetics of adaptive reorganization (i.e., the process of stabilization and maintenance of adaptation in the presence of the stimulus, and the further course of adaptation after disappearance of the factor which evoked it). The model we have chosen for adaptive reorganization has its analogies in spaceflight, in that there, along with conditions which frequently change, we meet a whole complex of factors which alter very little in their intensity, e.g., weightlessness, restriction of movement, increased partial pressure of CO_2.

As we have said already, the criterion of general adaptation was the organism's level of resistance and its susceptibility to the factor to which it adapted. Accordingly, the adaptability indexes in these experiments were taken to be the survival rate of the mice in a narcotic CO_2 concentration and their susceptibility to a given agent (determined from changes in the breathing rate and motor activity).

The experiments were conducted on laboratory mice weighing 18-25 g. The mice were placed in a 400 liter LK-NZh chamber, where a 7.5 percent CO_2 concentration was built up in 30-40 min. An OA-22-09 automatic gas analyzer maintained this concentration, while the oxygen content was varied between 18 and 30 percent. The control mice were placed in the same chamber, but in ordinary air. Survival tests were conducted during CO_2 exposure and for two weeks afterward. From 3 to 6 of the experimental animals were removed from the chamber and placed with a similar number of controls for 10-30 sec in a vented 3 liter vessel.

This vessel, which was hermetically sealed with a transparent cover, was filled with a gas mixture containing 70 percent CO_2 and 30 percent O_2. The time from start of pumping to moment of death (cessation of breathing) was de- /393 termined for each animal. The mean lethal exposure was determined for experimental and control mice in each test. During approximately the same periods a study was made with a unit employing closed recirculation of air (fig. 1). The entire installation, excluding the spirometer, was located in a water bath at a temperature of 22-23°C. Heat exchange was determined for 30 min. The animal was placed into a 150 cm^3 chamber through which, at a rate of 0.7-0.9 liter/min, CO_2-free air (CO_2 removed by passage through two vessels with a 0.1 N solution of NaOH) was pumped. The oxygen absorbed by the animals was replaced from the spirometer. The quantity of oxygen consumed could be judged from reduction of the water level in the lower chamber of the spirometer. The quantity of CO_2 excreted was determined by titration of the alkali residue with a 0.1 N solution of hydrochloric acid.

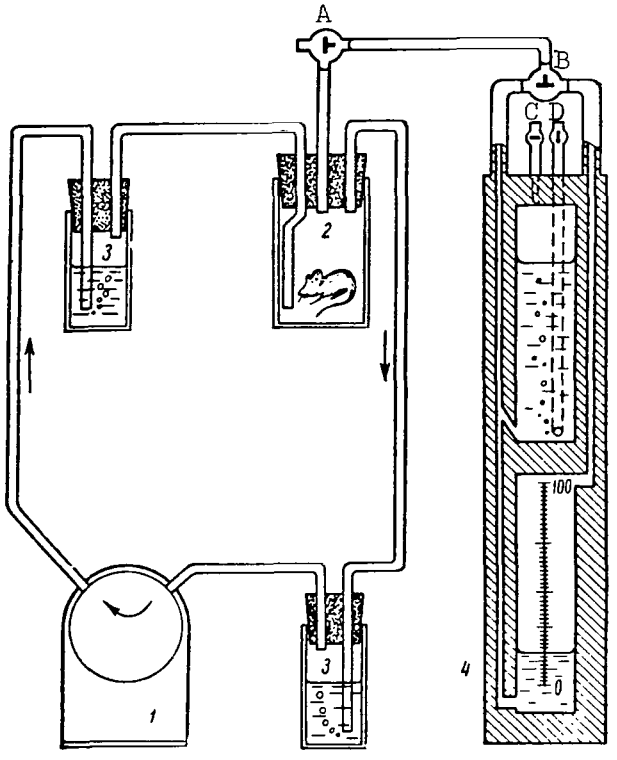

Figure 1. Device for studying gas exchange. 1, PR-7 air blower; 2, chamber; 3, vessels with 0.1 N NaOH; 4, double-chambered spirometer. Spirometer is filled with oxygen (through left outlet of valve A) after valves A, C and D have made 90° turn to left and valve B to right.

The mean arithmetic respiratory quotient was computed on the basis /394 of RQs obtained in separate measurements and not as the ratio of the mean arithmetic quantity of CO_2 liberated to that of O_2 consumed. Every two hours during exposure periods a 15 sec visual check was made of 4 or 8 mice for respiration frequency and motor activity. The latter index was taken as the ratio of "mobile" mice to the total number (10 or 20), expressed in percentage. "Mobility" was defined as one movement in a 10 sec visual observation. A statistical analysis was made by comparing the paired groups with Student's criterion (t). Empirical regression series (functional dependence of the degree to which the effects were manifested in the respiratory and motor apparatus on the length of exposure to 7.5 percent CO_2) were adjusted by the least squares method. The increase in breathing rate and the depression of movement were expressed in altered form, as the angle α, the arc sin of which equals the square root of the absolute value of the difference between experimental and control data (in percent)

$$\alpha = \text{arc sin} \sqrt{\left(\frac{\text{exper-control}}{\text{control}} \cdot 100\right)} \text{ percent.}$$

The survival rate of the mice in the 70 percent CO_2-30 percent O_2 mixture during the first hours of their exposure to 7.5 percent carbon dioxide did not change in essentials, apart from a certain tendency to foreshortening of life in a hypercapnic medium (fig. 2). Toward the end of the first days, however, a statistically significant increase was observed in the general resistance to the effects of this factor. In this period the lifetime of the experimental and control animals in the 70 percent CO_2 mixture was 83.8 ± 7.2 min and 45.4 ± 7.8 min, respectively. The difference between these values is 38.4 ± 9.6 min ($P < 0.05$). At the end of a two-day exposure, as well as during the succeeding 48 hours, the resistance of the experimental mice remained noticeably higher than that of the controls. However, statistical analysis showed this distinction to be insubstantial.

Subsequently, beginning with the third day after exposure, a different picture emerged. Between 72 and 96 hours a reduction in the strength of an organism subjected to 7.5 percent CO_2 became apparent. The difference in lifetime in 70 percent CO_2 between the control and experimental mice in this period was 19.1 ± 14.7 min. In the following days (120-192 hours after exposure), a statistically significant difference arose between the survival rate of the experimental and control mice (11.6 ± 3.7 min).

The results of a more prolonged, 120 hour exposure are presented in the table. They show that the resistance of the organism of the experimental mice during and after exposure to 7.5 percent CO_2 was modified in the same direction as during a 48 hour exposure: after disappearance of the given agent a certain increase in the resistance of the experimental mice compared to the control was noted; the reverse was true after a 120 hour exposure. /395

Data on changes in the reaction of the respiratory system and motor apparatus in the course of 48 hour exposure to CO_2 showed a marked increase in the breathing rate of experimental mice during the entire experiment. It is remarkable that during the first days the intensity of this reaction declined steadily, while subsequently respiratory frequency remained constant, exceeding by about 20 percent that of the control mice. The dependence of the rate of respiratory increase on the duration of exposure is described with sufficient precision by an asymptotic function (fig. 3), in which the correlation coefficient or value of the correlative ratio η is 0.821 ($P = 0.01$).

The motor activity of the mice throughout the experiment was reduced in comparison with the controls (lower half of table). The depression of movement had a marked tendency to decline: the coefficient of correlation equalled -0.539 ($P = 0.07$). /396

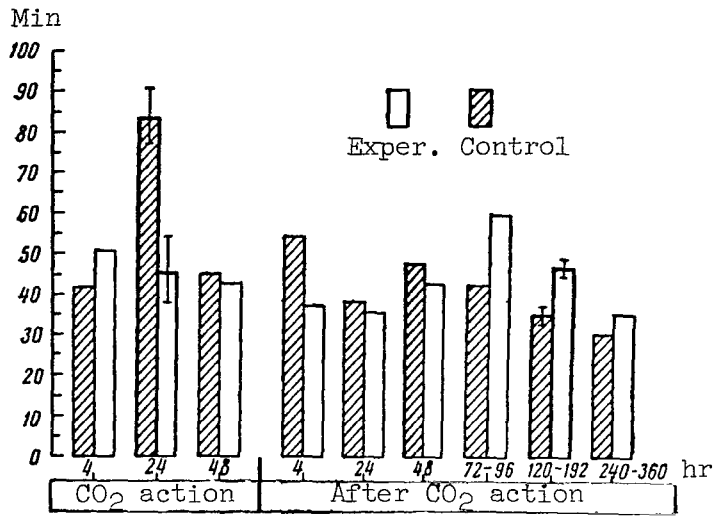

Figure 2. Lifetime of mice in hypercapnic mixture (70% CO_2) during and after exposure to atmosphere with 7.5% CO_2: vertical lines at tops of columns (mean average error) are provided for data indicating statistically significant difference between control and experimental animals ($P < 0.05$).

CHANGE IN RESPIRATION FREQUENCY AND MOTOR ACTIVITY OF MICE DURING EXPOSURE TO 7.5% CO_2: AVERAGE DATA OF FOUR EXPERIMENTS.

Exposure, hr		4	8	12	16	20	24	28	32	36	40	44
Number of respirations per min	Experimental	187	192	189	181	180	184	192	189	178	183	174
	Control	140	147	148	139	150	147	161	151	140	146	139
	Difference	+47	+45	+41	+42	+39	+37	+31	+38	+38	+37	+35
Percentage of animals found mobile	Experimental	23	22	28	26	22	23	33	31	34	25	24
	Control	37	34	53	32	42	30	46	46	38	31	28
	Difference	-14	-12	-25	-6	-20	-7	-13	-15	-4	-6	-4

Study of gas exchange showed that in the first hours of carbon dioxide exposure a tendency appeared toward increased respiratory quotients (fig. 4). At the end of the first hours of exposure there was a drop in the quantity /397 of oxygen consumed and of CO_2 excreted, while after 48 hours a decline in CO_2 liberation was observed (from 4.22-3.92 ml/g·hr). However, all these shifts proved insignificant under statistical analysis ($P \geq 0.10$).

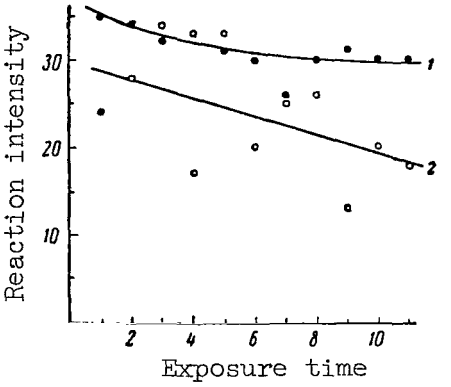

Figure 3. Changes in relative magnitude of reaction of respiratory system (increase in breathing rate) and in motor apparatus (depression of motor activity) at various periods of exposure of mice to 7.5 percent CO_2: each

unit of abscissa equals 4 hours; each unit of ordinate denotes reaction intensity in angles

$$\alpha = \text{arc sin}\sqrt{\left|\frac{\text{exper-control}}{\text{control}}\right| \cdot 100)} \text{ percent}$$

1, respiratory system reaction; 2, motor apparatus reaction.

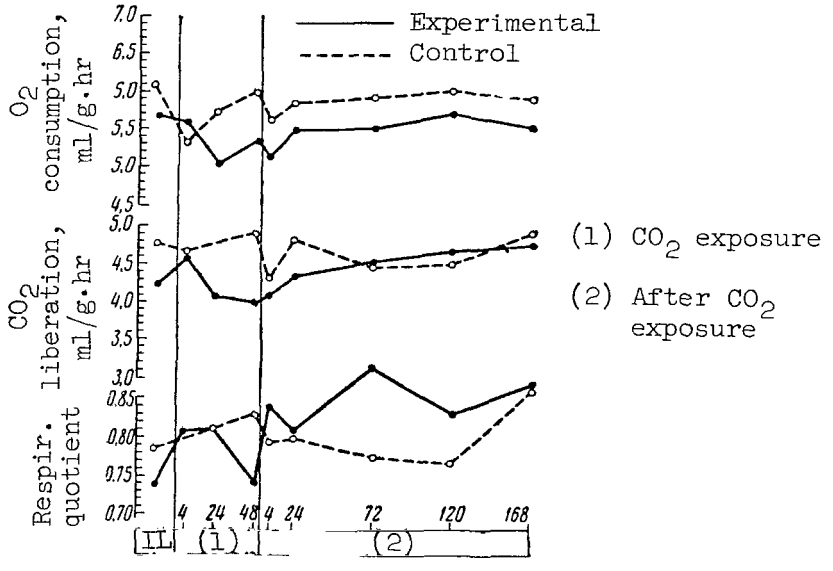

Figure 4. Dynamics of gas-exchange indexes of mice during and after exposure to 7.5 percent of CO_2:

small circles indicate difference between control and experimental mice ($P < 0.05$); IL, initial level.

With respect to the respiratory quotient, changes with greater definition appeared at the end of exposure. The difference between the value of the RQ in experimental and control mice at that time averaged 0.094 ± 0.033 ($P < 0.05$). Before CO_2 exposure (fig. 4) this difference was only 0.051 ± 0.047 ($P > 0.30$).

Consequently, disregarding the fact that after a 48 hour exposure the RQ was at approximately the same level as before, it was markedly less than that of the rising quotient of the controls during this period.

During the aftereffect period the consumption of oxygen and the excretion of CO_2 showed no important changes. There was only a certain tendency to an increase in CO_2 excretion (72 and 120 hours after exposure) and an equally insignificant drop in oxygen consumption (4, 24, and 72 hours after 7.5 percent CO_2 exposure). Quite well-defined shifts were found in the respiratory quotient.

It should be noted that afterwards the RQ changes had the reverse sign from those recorded at the end of exposure. In the first hours after cessation of 7.5 percent CO_2 exposure the difference between the RQ of the experimental and control mice was $+ 0.048 \pm 0.017$ ($P < 0.05$), while after 72 hours it had become more palpable: $+ 0.112 \pm 0.040$ ($P < 0.05$). After 24, 120 and 168 hours following exposure the magnitude of the respiratory quotient of the experimental mice was also higher than the initial level and the corresponding level of the controls, although from a statistical point of view these displacements were not significant.

The study of gas exchange thus permits us to discover a clearly defined change in the respiratory quotient: an increase in the RQ at the end of an exposure of 48 hours and a long-lasting, or at least twofold, increase after action by 7.5 percent CO_2. With respect to other gas-exchange parameters we can speak only of certain tendencies, as, for example, of a reduction in energy transfer at the end of the first days of exposure, and so forth. /398

These results provide grounds for asserting that in the course of exposure to carbonic acid the mice adapt to that agent, inasmuch as their functional state changes in the direction of increased resistance and decreased susceptibility to CO_2.

On the basis of the data obtained, discussion could continue treating specific adaptation; but at the same time, apparently, resistance to any harmful agent is increased. This is supported by Walker (ref. 18), whose data show that in the case of mice who have adapted over 10-20 days to life in a medium with 10 or 20 percent CO_2, the spasms caused by high partial pressure of oxygen (10 atm) begin considerably later than in control animals who have not adapted.

We should focus attention on the fact that authentically heightened resistance of the animals was observed only in the first days, while later, at the end

of a 48 hour exposure, this effect had abated. Two propositions can be made from this: either the phenomenon of adaptation discovered in our experiments disappears rapidly, being replaced by Selye's "exhaustion phase"; or else we are dealing with an oscillation of the adaptation process.

Two factors speak against the first proposition: on the one hand, data in the literature testify to prolonged maintenance of an adaptive state in mice exposed to higher concentrations of CO_2 than those in our tests (ref. 18), while on the other hand there is the repeated increase in survival rate, revealed in our experiment, after a 96 hour exposure. Consequently, we must consider the oscillatory development of adaptation. It is entirely possible that this is a characteristic feature in establishing adaptation in the experimental model given here and in its analogs, for cases in which changes in external conditions occur rapidly, with pronounced gradients. On the same theme we must emphasize that the absence at the end of two days of increased resistance to the effects of high CO_2 concentration was observed simultaneously with signs of a drop in the susceptibility or reaction of certain physiological systems to the "agent being adapted to," the 7.5 percent CO_2; i.e., there was a tendency to continuous weakening of the reaction of the respiratory system and the motor apparatus during the entire exposure period. We can thus reckon that even at the end of two days of CO_2 exposure there occurred not a disappearance, but merely a weakening of the adaptive condition.

It seems to us that another fact is deserving of special attention-- /399 the decline in the resistance of the animals to CO_2 several days after the end of the exposure period. Similar phenomena are described in the literature on the basis of studies of the so-called "entrance" and "exit" phases of poisons (morphine, nicotine, etc.) during tests on isolated organs (ref. 6), as well as of the reaction of an entire organism to cessation of action of habitual or long-acting stimulants (light, sound, etc.), both in chronic and acute experiments (refs. 5, 6, 10 and 15).

To strike a balance in this discussion devoted to the identification of the two basic phenomena uncovered in our study, it is appropriate to suppose that the increased stability of the organism of mice during CO_2 exposure, and the opposite condition following several days after its cessation, are in all probability one of the general laws of adaptive reorganization in living systems. These laws, evidently, are a reflection of such peculiarities in the kinetics of transitions into new static states (under the action of an "unusual" or the disappearance of a "habitual" agent) as are present only in homeostatic open systems, to which living organisms belong (refs. 4, 11 and 12).

With respect to the mechanism of adaptive reorganization, the results of this experiment permit us to asseverate only propositions about the possibility that metabolism participates in these reorganizations. The ground for this

supposition is the reliably established drop in the respiratory quotient at the end of exposure and the increase which follows. Inasmuch as the RQ reflects quantitative relations of nutrient substances included in energy transfer, we assume that during and after exposure to CO_2 certain changes occur in the bio-

catalytic processes at the substrate-oxidation level. Along with this we must take into account shifts in electrolyte exchange connected with pH changes and in the blood's alkali reserve (refs. 3 and 16), as well as the role of the kidneys in the excretion of CO_2 during increased concentrations of carbon dioxide

in the atmosphere (refs. 1 and 16).

Reorganization of the enzyme systems, just as in the case of establishment of a new electrolyte-exchange level, requires considerable time. According to the data of reference 16, for example, compensation for disturbances to the acid-alkali equilibrium in test animals subjected to prolonged 2-3 percent CO_2

concentrations takes place only in the third week after cessation of exposure. Therefore, the long-lasting increase in respiratory quotient should cause no astonishment, nor should the tardiness of several days in the phenomenon of reduced survival rate among the mice after cessation of exposure to 7.5 percent CO_2. A more precise understanding of the reasons for such prolonged aftereffect,

and determination of the role of the nervous and endocrine systems in reactions to CO_2, can be achieved only in special studies of the physicochemical and

enzyme reorganizations mentioned above. Such studies are important, both /400
in practice to determine permissible CO_2 concentrations and to define more pre-

cisely the dynamics of the respiratory quotient, which require strict accounting in the creation of air-regeneration systems for spacecraft.

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INJURIOUS EFFECT OF 660 AND 120 MeV PROTONS AND THE EFFICACY OF PHARMACOLOGICAL AND CHEMICAL PROTECTION

V. S. Shashkov and V. S. Morozov

We may arbitrarily divide the spaceflight factors which affect /401
living organisms into three basic groups. The first contains those related to the dynamics of flight in spacecraft (noise, vibration, acceleration, weightlessness); the second, those which characterize outer space as a special environmental medium (various kinds of photo- and ionizing radiation, peculiarities of thermal conditions, etc.), and the third, those connected with the more or less prolonged sojourn of the organism in the closed space of the craft (ref. 1).

One of the most difficult problems in the path of further conquest of space is the danger of injury from the effects of ionizing radiation, particularly during long spaceflights and flights in the radiation belts of Earth (refs. 2 and 3).

According to present-day concepts, cosmic radiation includes rays originating within our galaxy (primary cosmic radiation), protons of various energies formed during solar flares and penetrating radiation from near-space.

From the practical viewpoint of radiation danger, special interest attaches to the corpuscular radiations (high-energy protons and heavy, multicharge nuclei) which are formed in great quantities during chromospheric eruptions on the Sun, and the penetrating radiations of Earth's inner and outer radiation belts.

In view of the complexity of the spectrum of cosmic radiation, the inexactitude of computing absorption doses for high-energy particles and the inhomogeneous transformation of tissues by absorbed energy which is scattered through ionization, electron excitation, nuclear interaction, stimulation of /402
X- and gamma bremsstrahlung, etc., determination of the biological effectiveness of the dose and the components of cosmic radiation present considerable difficulties.

What hinders us most in developing an approximate evaluation of the effects of cosmic radiation, when sufficient information concerning its physical constants and satisfactory dosimetry are at hand, is the lack of the parameters of relative biological effectiveness (RBE) for cosmic radiation.

Investigation of the RBE--and especially the protons, neutrons and heavy nuclei--is one of the basic radiobiological problems of spaceflights, taking on even greater significance as spacecraft are equipped with nuclear-power engines.

This problem received more intensive study as high-altitude balloons, rockets and especially satellites and spacecraft which could return to Earth made it possible to conduct onboard experiments on the biological effects of cosmic radiation. The trouble with such experiments is that the radiation effects had to be isolated from a whole complex of spaceflight factors.

The result has been a race back to the laboratory to reproduce recorded levels of radiation; but this procedure is often attended with additional constraints.

Laboratory determination of the heavy component RBE is not possible at present, because there are no devices to generate particles of such high energy. Even if they existed, they would be unsuitable for biological experimentation.

The obstacle here is that even given data on the RBE of all cosmic radiation components it is still very difficult to form a judgment regarding the biological effects of radiation of complex spectrum (refs. 4-6). Obviously, these goals demand the design of special installations, delivering a mixed beam of predetermined spectrum and known energies of the separate components.

As we noted, the RBE of various types of radiation, including components, depends on an assortment of factors, among which ionization density and linear losses play a large role. Thus, increasing specific ionization from 3-100 ion pairs per micron of path finds little reflection in RBE, which remains at approximately one unit. An increase from 100-1,000 raises the RBE in /403
proportion to the logarithms of specific ionization (ref. 7).

Various authors (refs. 7-14) have calculated the RBE from 2-30 for specific ionization higher than 1,000 ion pairs/micron.

At the present point in time the first steps have been taken in laboratory identification of the RBE of protons. According to data of E. B. Kurlyandskaya (refs. 15 and 16) and G. A. Avrunina (ref. 17), the RBE of 660 MeV protons relative to Co^{60} gamma radiation is 0.5-0.6 for mice and 0.6-0.7 for rats. For 510 MeV protons A. V. Lebedinskiy, Yu. G. Nefedov, N. I. Ryzhov et al. (ref. 18) have established an RBE of 1.2 for dogs and 0.8 for rats.

In experiments made to compare 730 MeV proton RBE in monkeys against that for gamma rays, a value of about 2 was obtained (ref. 19); in an experiment to compare 157 MeV protons with X-radiation, the RBE for mice turned out to be 0.77 ± 0.1 (ref. 20).

It is important to note, in citing data from the literature, that the RBE of any kind of radiation depends on what symptom was taken as a base. The RBE for one and the same kind of radiation often differs for various biological systems and organs (refs. 8-10, 13, 14 and 20-35).

Thus, radiation injuries caused by protons and neutrons is more irreversible than those caused by action of X-rays. With respect to its genetic effect and injury to the crystalline lens, a certain cumulativeness has been discovered in corpuscular radiation. During an increase in radiation time and observation period after radiation by high-energy particles the RBE rises substantially (refs. 4 and 6).

Neutron radiation causes more pronounced changes in the chromosome apparatus than in cell structures unrelated to them. For X-rays this relationship is reversed (refs. 25, 26 and 34).

The RBE also depends on the radiation dose, the functional condition of the organism, etc. The irreversibility of changes brought about by corpuscular radiation must be kept in mind in studying the biological effects of cosmic radiation having a heterogeneous, complex character (ref. 26).

In evaluating the genetic effects of cosmic radiation we must consider both the danger to offspring and the somatic and cytogenetic alterations of the irradiated organism, which leads to foreshortening of life, blood diseases, malignant growths and other pathological phenomena (refs. 32 and 33).

While recognizing that under laboratory conditions we are dealing with particles emitting separate cosmic radiation components, we nevertheless /404 to some extent can evaluate their biological effectiveness by using installations which generate various types of radiation.

In the present report we studied the effect of protons with energies of 120 and 660 MeV on animals and the prophylactic action of substances effective against X- and gamma-radiation.

The experiments were conducted on 1,360 desexed white male mice weighing 18-21 g. The animals were exposed to gamma radiation from a Co^{60} source and to 120 and 660 MeV protons.

The animals were subjected to gamma irradiation on the GUBE-800 experimental unit of the Biophysics Institute AN SSSR at a dosage of 264 r/min. Proton irradiation was accomplished in a pulsed beam of the Joint Institute for Nuclear Research at Dubna; protons were used in a density of 10^8-10^9 per cm^2/sec.[1]

The quantity of pulse was 100 per sec, with a pulse length of 200-400 m/sec. Determination of 660 MeV proton dosage and irradiation were conducted with the method described by Avrunina (ref. 17). Dose power, determined from the induced activity in carbon plates, was 500-700 rad/min in our tests. In all experiments the animals were irradiated in plastic chambers, 10 at a time (5 controls and 5 given prophylactic substances).

[1]We take this occasion to express our gratitude to Corresponding Member AN SSSR D. I. Blokhintsev, Director of the Joint Institute of Nuclear Research; and Professor V. P. Dzhelepov, director of JINR's Nuclear Problems Laboratory, for making possible the proton experiments.

We point out that the data vary depending on the dose value corresponding to 1 proton. Avrunina, on the basis of calculations made with Komochkov (ref. 17), found that the dose for 1 proton with an energy of 660 MeV was $(r.3 \pm 0.18) \cdot 10^{-8}$ rad.

Staff members of the Institute of Biophysics, Academy of Medical Sciences, obtained an analogous value of $4.3 \cdot 10^{-8}$ rad/proton.

Foreign data (ref. 35) give a value of $5 \cdot 10^{-8}$ rad for a 500 MeV proton. In our tests a magnitude of $4.3 \cdot 10^{-8}$ rad/proton was obtained.

Reduction of proton energy from 660 to 120 MeV was accomplished by polyethylene blocks 1.5 m thick with an additional 6 cm layer of lead. Current intensity and proton dose were determined from induced activity in carbon indicators, the counting errors of which were conducted by a Type B unit with an MST-17 ring counter. Fluctuations in beam intensity were monitored. Dose power at 120 MeV was 60 ± 10 rad/min.

Determination of proton RBE was not included in our program. However, having at our disposal a considerable amount of animal control groups, we had the opportunity of obtaining RBE data. For 660 MeV protons we /405
derived the RBE from the effects of various radiation doses on the longevity of the animals during a 30 day observation, as well as from weight changes.

The data of these experiments are presented in table 1.

As table 1 shows, for Co^{60} gamma rays $LD_{50/30} \sim 650$ r (600 rad); for 660 MeV protons, 900 rad. $LD_{100/30}$ in relation to Co^{60} gamma rays was 850 r (790 rad); in respect to 660 MeV protons, 1170 rad. The RBE of protons, consequently, is 0.73 for LD_{100}. These data are close to those of Avrunina's experiments, if we take into account the difference in initial values for the dose per proton.

It is well known that the RBE of various types of ionizing radiation depends on the density of ionization created by particles in a substance along the path of their movement, i.e., on the linear energy losses (LEL).

During interaction of 660 MeV protons with a substance a complex spectrum of secondary particles is formed, part of which possess an LEL considerably higher than that of the protons. Their contribution to the injurious /406
effect is apparently to a large extent found in reckoning the remote sequelae, judging from which the proton RBE is not less than one unit.

The problem of chemical and biological protection against ionizing radiation comprises a special branch of radiobiology, radiation medicine and space radiobiology. A. A. Gyurdzhian (ref. 36), referring to data in the literature, is inclined to believe that various chemical substances, which are effective for

TABLE 1. RBE FOR VARIOUS DOSES OF 660 MeV PROTONS IN RELATION TO Co^{60} GAMMA RAYS (rad).

Co^{60} gamma rays					660 MeV protons						
Dose, r	No. of animals	Survived to 30th day	Survival, percent	Average life-time, days	Dose, rad*	No. of animals	Survived to 30th day	Survival, percent	Average life-time, days	LD: gamma protons	RBE LD_{50} and LD_{100}
400	40	40	100	-	400	40	40	100	-	-	-
500	40	28	80	18.6	500	40	40	100	-	-	-
-	-	-	-	-	560	40	38	95	12.8 ± 5.3	-	-
600	40	24	60	12.1	650	40	32	80	14.2 ± 4.2	40/20	-
700	40	16	40	11.8	750	40	20	70	14.1 ± 3.2	60/30	0.75**
800	40	6	15	8.4	850	40	22	55	16.3 ± 3.1	85/45	-
-	-	-	-	-	950	40	12	30	12.4 ± 3.3	-/70	-
850	40	0	0	7.8	1178	160	3	1.7	11.7 ± 2.9	100/98.3	0.73***

*To convert r into rad the following coefficients were used: 1 r = 0.93 rad; 1 r = 98 erg/g; 1 rad = 100 erg/g

**LD_{50}

***LD_{100}

X- and gamma radiation, are ineffective for corpuscular radiations, especially with respect to their genetic influence.

It is also generally known that when using antiradiation agents to protect against high-density ionization there is a reduction in their activity for neutron radiation (refs. 37 and 38), which may become a total absence of activity in the case of alpha radiation (ref. 5).

However, as was shown for the first time by S. P. Yarmonenko, G. A. Avrunina, V. S. Shashkov, R. D. Govorun (ref. 39) and S. P. Yarmonenko (ref. 40), the prophylactic action of familiar radioprotective agents during 660 MeV proton irradiation is just as great, if not larger, than during X- or gamma irradiation from a Co^{60} source.

The most convincing data on the question of the comparative radioprotective effect of prophylactic substances during gamma or proton irradiation have been presented by S. P. Yarmonenko (ref. 40).

We tested the following substances during Co^{60} gamma irradiation and radiation by protons with an energy of 120 and 660 MeV: cystamine dihydrochloride

TABLE 2. PROPHYLACTIC EFFECT OF PHARMACOCHEMICAL SUBSTANCES DURING Co^{60} GAMMA IRRADIATION IN AN 850 r DOSE ($LD_{100/30}$).

Preparation	No. of mice	Survived to 30th day	Survival percent	Average life-time, days
Cystamine	40	22	55	13.8 ± 3.7
AET	40	30	75	15.3 ± 4.2
Serotonin	40	24	60	12.4 ± 3.5
5-methoxytryptamine	40	28	70	14.9 ± 3.7
Tryptamine	20	8	20	8.3 ± 3.3
5-oxytryptophan	20	8	20	8.0 ± 3.5
Control	40	0	0	7.5 ± 2.8
Biological control		20	100	30.0

(150 mg/kg); S, β-aminoethylisothiuronium (AET - 150 mg/kg); serotonin creatinine sulfate (50 mg/kg); 5-methoxytryptamine hydrochloride (75 mg/kg); tryptamine hydrochloride (100 mg/kg), and 5-oxytryptophan (250 mg/kg). /407

All preparations were introduced intra-abdominally 10-15 min before radiation. Preparation doses were taken into account in calculating the base.

The data of these tests are presented in tables 2, 3 and 4.

Table 2 shows that the greatest radioprotective activity against Co^{60} gamma radiation in an absolute lethal dose was afforded by AET, 5-methoxytryptamine, serotonin and cystamine. The protective action of tryptamine and its derivative, 5-oxytryptophan, which synthesizes serotonin in the organism, is considerably less.

Data on the radioprotective action of substances during total irradiation with 660 MeV protons are presented in table 3.

In analyzing the results of the experiments recorded in table 3, we see that during 660 MeV proton irradiation the most effective prophylactic agents, just as in the case of gamma radiation, are AET and 5-methoxytryptamine. The protective effects of cystamine and serotonin during proton exposure are analogous to their prophylatic properties during gamma radiation. It is interesting to note than in Yarmonenko's experiments (ref. 40) the high degree of protection afforded by AET is conserved during irradiation with protons in hyperlethal doses (1,600 rad).

TABLE 3. PROPHYLACTIC EFFECT OF PREPARATIONS DURING TOTAL IRRADIATION OF MICE BY 660 MeV PROTONS IN A DOSE OF 1170 ± 150 rad ($LD_{\sim 100}$)

Preparation	No. of mice	Survived to 30th day	Survival percent	Average life-time, days
Cystamine	80	41	51.2	15.3 ± 3.8
AET	60	49	81.2	14.5 ± 2.9
Serotonin	30	15	80.0	12.8 ± 3.5
5-methoxytryptamine	30	21	70.0	14.2 ± 3.3
Tryptamine	20	5	25.0	10.9 ± 2.4
5-oxytryptophan	20	4	20.0	11.0 ± 2.4
Control	160	3	1.7	11.7 ± 2.9
Biological control	60	59	98.4	24.0

TABLE 4. PROPHYLACTIC EFFECT OF PREPARATIONS DURING TOTAL 120 MeV PROTON IRRADIATION IN DOSES OF 1200 ± 100 rad ($LD_{\sim 100}$).

Preparation	No. of animals	Survived to 30th day	Survival percent	Average life-time, days
Cystamine	40	24	60	13.5 ± 4.3
AET	40	30	75	16.3 ± 4.6
Serotonin	40	22	35	12.7 ± 3.5
5-methoxytryptamine	40	28	70	13.8 ± 3.2
Control	60	2	3.3	9.5 ± 3.1
Biological control	20	20	100	30.0

We found no data in the literature on the radioprotective effects of common effective antiradiation agents during 120 MeV proton irradiation of animals. The results of our experiments with protons of this energy are reproduced in table 4.

Table 4 shows that the effectiveness of the radioprotective prep- /408
arations we have studied, which possess antiradiation properties for X- and gamma radiation and 660 MeV proton irradiation, is fully retained for 120 MeV protons.

In summarizing the results of these experiments it is possible to conclude that the RBE for mice of 120 and 660 MeV protons compared with electromagnetic radiation is no greater than 1 and can be even less. The main point is that agents commonly employed as prophylaxis against X- and gamma radiation retain their effectiveness against high-energy proton radiation.

We are not concerned here with the mechanisms by which the radioprotective effectiveness of pharmacochemical agents, which has not been explained up to now, is accomplished.

The presence of protective properties of a number of chemical substances during corpuscular radiation justifies the quest for a means of chemical and biological protection of man and the entire biocomplex of a spacecraft. This requires further investigation of the principles of pharmacochemical protection under various energies, doses and intensities of corpuscular radiation (especially protons) and study of the effects of radiation in combination with other factors of spaceflight. This creates the possibility, however limited, to evaluate radiation dangers, determine permissible levels of cosmic radiation and take effective measures to guard against it.

In this connection the combined use of radioprotective agents with /409
deliberately chosen different mechanisms of protective and pharmacological effect acquires considerable interest and practical importance. It is of no little importance that by using a series of low-toxicity pharmacological preparations, which have no negative effect on the antiradiation properties, it is possible to increase their prophylactic effect after reducing their toxicity.

For this reason a more profound investigation of the pharmacodynamics of separate radioprotective agents and their carefully considered combination is of foremost importance in developing new radioprotective preparations intended for practical use.

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EFFECT OF SCREENING INDIVIDUAL PARTS OF THE BODY OF ANIMALS ON CHANGES IN RADIATION REACTION AND EXPOSURE TO GAMMA RAYS AND HIGH-ENERGY PROTONS

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During the two decades which have passed since mankind, as a result /411
of the use of the atomic bomb, first encountered radiation injuries of a mass character, the attention of scientists has more and more been drawn to the effects of ionizing radiation on living organisms and to the search for methods of reinforcing the reaction of the organism to radiation. The great importance of studying these problems has led to a continuous increase in the number of persons who, by virtue of their professions, are constantly exposed to various types of ionizing radiation. Man's penetration of space is attended by the dangers of cosmic radiation, the basic component of which ($\sim$ 85 percent) is composed of high-energy protons. So long as flight orbits remain below the radiation belts this danger is not great; however, prolonged interplanetary flights sharply increase the probability of dangerous radiation doses, especially from high-energy protons emitted during flares on the solar chromosphere (refs. 4 and 5).

During the last 15 years, both at home and abroad, reports have been published dealing with the effects of shielding separate organs or areas of the body on the reaction of animals exposed to radiation. A number of studies employed shielding to alter the total body radiation reaction of the /412
organism, reducing the severity of its manifestations. In other reports shielding was used to isolate separate components of the radiation reaction for the purpose of studying various symptoms of this complex process and discovering the mechanisms of its development.

Jacobson and his associates in 1949 (refs. 14 and 15) were the first to demonstrate the possibility of altering the reaction of animals to total body irradiation by the use of shielding. A lead-box shield around the spleen of mice subjected to 600 to 1,050 r X-ray doses resulted in a pronounced mitigation of the severity of radiation injuries and reduced death rate by comparison with the controls. The lethal dose was approximately 50 percent higher (LD_{50}) for animals with spleen-shielding than in those without. In Jacobson's opinion, this effect is explained by the capacity of the spleen to compensate for rapidly disturbed hematopoiesis in any organ.

In recent years Jacobson (refs. 16-19) and other investigators (refs. 2, 3, 6-11, 20 and 21) have published a series of reports in which they have presented the results of their studies of the effects of shielding of various

parts of the body on the progress of radiation reaction in laboratory animals (chiefly mice and rats) at various radiation doses. It has been established that rats, as distinct from mice, derive a more beneficial effect from shielding the bone marrow than shielding the spleen. Generally, however, shielding sections of the blood-building organs has an appreciable mitigating effect on radiation reaction only within a definite dose span, approximately 1,000-1,200 r. At higher doses, shielding the small intestines will have a substantial influence on the course of radiation reaction, especially in combination with protection of the bone marrow, which is best accomplished by placing a screen of protective material in the region around the upper half of the abdomen.

Disregarding the various results obtained by different authors, a basic conclusion, not open to doubt, emerges from these investigations: shielding various organs or areas of the body during total body X-ray or gamma irradiation of animals is an effective method of weakening the manifestations of radiation reaction, which includes reducing the frequency of fatal sequelae after administration of large radiation doses.

In the present work some data are presented concerning the influence of shielding various sectors of the body on radiation reaction during total body irradiation of rats by gamma rays and 120 MeV protons. We know of no studies on the effects of shielding for high-energy particles, among them protons.

All experiments were conducted on white, desexed rats of both /413
sexes, weighing 175-200 g. Prior to the experiment the rats were examined, marked and weighed two or three times. An examination of the blood was made for part of the animals, taken from the tail vein. After irradiation a daily record was kept of how many rats died, and the survivors were weighed regularly. Blood examinations were made in hematological groups on the 3rd, 7th, 15th and 30th days after irradiation. The overall control period was 30 days.

Gamma irradiation of animals was conducted on an experimental irradiator with a charge of Co^{60} at a distance of one m and a dosage strength of 15.5 r/min. Dosimetry of γ-irradiation was performed by usual methods.

The Joint Institute for Nuclear Research at Dubna provided the synchrocyclotron by which the animals were irradiated with a proton flux. The accelerator emitted a narrow beam of protons with an energy of 657 ± 5 MeV in the form of pulses with a duration of 100-400 μsec at a rate of 100 pulses/sec. Proton energy was reduced from 600 to 120 MeV by polyethylene blocks 150 cm thick reinforced with 6 cm of lead. Replacing the polyethylene with lead at the point of beam exit from the blocks permitted us to a certain extent to broaden the effective cross section, increase the number of animals irradiated simultaneously and reduce the differences in the dose received by each group.

The proton flux density and dose were determined from induced activity in carbon indicators, calculation errors of which were corrected with a Type B device with an MST-17 annular counter. Fluctuations in beam intensity were monitored. Dose power for 120 MeV protons was 60 ± 10 rad/min.

More detailed technical specifications on the accelerator and the nature of the dose field are given in a paper by V. P. Afanas'yev, I. B. Keirim-Markus et al. (ref. 1).

Prior to irradiation the rats were fixed to slabs by soft tapes. During proton irradiation the slabs with the rats were placed vertically on special stands on which 12 animals could be irradiated simultaneously. In experiments with gamma rays only 6 rats were taken at once.

Steel plates of various widths and about 15 cm thick were used to shield the parts of the body during gamma irradiation. This brought about a sharp reduction in radiation dose behind the screen and in the total dose absorbed by the entire body. In radiating rats with protons we employed polymethylmetacrylate (plexiglass) blocks 2-6 cm wide and 12-15 cm thick. These blocks, practically speaking, completely cut out part of the proton flux falling on the shielded area of the body, which naturally reduced the total dose absorbed by the tissues of the irradiated animals. To determine the magnitude /414 of reduction in the absorbed dose we must determine the mass of the tissues located behind the screen, as well as their relative size, in percent, with respect to the mass of the body of the rat. To this end we weighed the shielded parts of frozen carcasses of the rats. The results of these measurements are presented in table 1.

Determination of the mean tissue dosage, in ergs per gram tissue, entering the whole body at a given radiation dose and various areas of body protected by shielding can be made from the graph (fig. 1), where the abscissa shows the percent of the body which was shielded and the ordinate shows the mean tissue dose (in erg/g).

Study of the results of experiments by other authors has shown that the great effect on the development of radiation reaction during total body irradiation of animals is afforded by shielding parts of the abdomen, especially the upper section. As our measurements showed, in order to shield two-thirds of the abdomen, beginning from the sternal margin, it is necessary to employ a screen about 6 cm wide, which protects the entire liver, a large part of the spleen (and often the entire spleen) and a considerable part of the intestines. Besides, various bone marrow sections are screened (vertebrae, lower ribs), as well as the stomach and certain other organs of the pelvic girdle. In a series of experiments screens 2 and 4 cm wide were used. All numerical experimental material underwent statistical treatment.

Our basic experiments were devoted to a fairly detailed study of /415 the effects of shielding the abdomen on the course of radiation reaction during total body irradiation of the animals by gamma rays and protons. The reason for our investigating localization of protective shielding was given by data in the literature on the great effectiveness of shielding this region of the body during total body X-ray irradiation of animals. However, the complete absence of any kind of experimental studies on the effect of shielding on the radiation reaction during total body irradiation of animals with high-energy particles, particularly protons, served as a basis for conducting a series of preliminary experiments to compare the effectiveness of shielding various parts of the body during proton irradiation. At the same time comparative tests were made with gamma irradiation of the animals. The basic parameter for evaluation of the effect was the survival rate over a 30 day observation period.

TABLE 1. ABSOLUTE AND RELATIVE WEIGHT OF SEPARATE PARTS OF BODIES OF RATS (average figures from 10 measurements).

	Body weight before dissection	Total weight of body parts after dissection	Weight of various parts of body					
			Head and upper extremities (without shoulder girdle)	Sternum region	Upper half of animal			Lower part of body
					Complete protection (6 cm)	2/3 protection (4 cm)	1/3 protection (2 cm)	
Average weight in g	202.25	201.7	27.6	40.85	73.1	53.0	24.35	60.15
Relative weight in % of total body weight	100	99.7	13.7	20.2	36.1	26.2	12.0	29.7

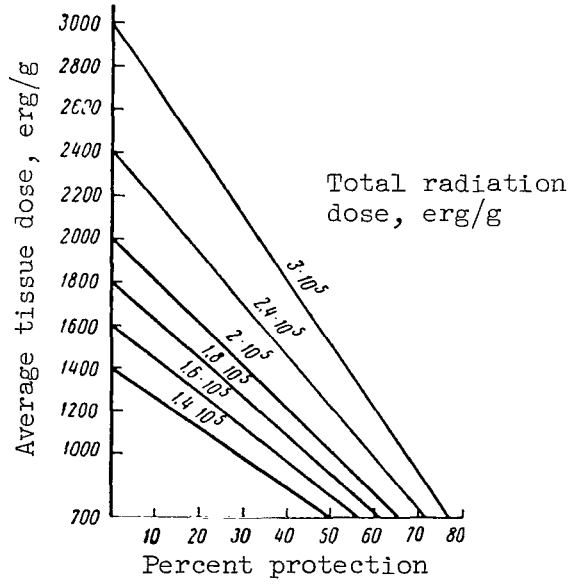

Figure 1. Dependence of average tissue dose on amount of shielding (in percent).

In the proton experiment we made a comparison of the effect of shielding individual masses of tissue of the head and abdomen. As is evident from table 1, when the entire head was screened, along with part of the extended upper extremities and the abdomen, with a 2 cm shield placed immediately above the umbilical line, the masses of shielded tissues were approximately the same: 13.7 and 12 percent of total body mass, respectively. During gamma irradiation we compared the effects of shielding three parts of the body: the head and abdomen under the same conditions, and also both haunches (along with the lower part of the pelvis) with a screen 4 cm wide.

The results of the experiments (in terms of survival rate) are presented in table 2. As we see, during total body irradiation of rats by protons in an 800-1,050 rad dose (LD_{80} in the control group), shielding the head yielded no increase, while shielding the abdomen led to a reliable increase in the survival rate of the animals as compared with the control group (up to 50 /416 percent). We must note that this experiment was conducted on a comparatively limited number of animals. In addition, a relatively broad dose spectrum was used to irradiate the various groups of animals. Therefore, we must be most cautious in the evaluation of these results. From data in the literature we know that shielding the head during X-ray irradiation of animals usually yields a certain increase in survival rate.

In the experiment with total body gamma irradiation of rats in a 930 rad dose (LD_{95} in the control group), using the same localization of protective blocks, we obtained a striking increase in the survival rate of the animals, not only with abdominal shielding, but also with shielding of the haunches and head. All three localizations gave reliable increases compared with the control group. However, the most pronounced effect occurred in abdominal shielding. Head and haunch shielding increased the survival rate to 30 and 44.8 percent, respectively, as compared with a control figure of 6.3 percent, while abdominal shielding produced an 80 percent survival. These differences between the abdominally shielded group and both the controls and the head/haunch-shielded group are reliable.

Notwithstanding a certain difference in results obtained in the two series that were run, we have every reason to conclude that during total body irradiation of animals with gamma rays and protons (just as in the case of X-ray irradiation reported by other authors) abdominal shielding is the most effective in reducing the death rate.

Even in the first experiments we noted a less severe radiation-sickness process among the rats whose bodies were partly shielded, especially the abdomen. Thus, for example, in an experiment with proton irradiation of the animals, a sharp drop in leukocyte count was observed in all three experimental groups on the 3rd and 7th days after irradiation. However, whereas the leukocyte count in the control group dropped to as much as 10.9 percent of the initial level, and in head-shielded rats to 14.4 percent, in rate with abdominal shielding the reduction was much less, only 29.1 percent of the initial level. This result is statistically reliable.

TABLE 2. EFFECT OF SHIELDING VARIOUS SECTORS OF BODY ON SURVIVAL RATE OF RATS.

Type of radiation and dose in rad	Area of body shielded	No. of rats	No. of rats surviving	Survival rate in % (M ± m)	Reliability of difference in survival rate with abdominal shielding		
					To radiation control	To head shielding	To haunch shielding
Gamma rays, 930*	Radiation control	48	3	6.3 ± 3.5	-	-	-
	Head	30	9	30.0 ± 8.4	$p<0.02$	-	-
	Both haunches	29	13	44.8 ± 9.2	$p<0.001$	-	-
	Abdomen (2 cm)	30	25	88.3 ± 6.8	$p<0.001$	$p<0.001$	$p<0.001$
120 MeV protons, 800-1,050	Radiation control	72	14	19.4 ± 4.7	-	-	-
	Head	24	4	16.6 ± 7.6	-	-	-
	Abdomen (2 cm)	24	12	50.0 ± 10.2	$p<0.01$	$p<0.02$	-

* 1 r = 0.93 rad

In the experiment with total gamma irradiation the data regarding changes in body weight in various groups are of interest. All rats, both in the control group and in those with shielding of various parts of the body, exhibited a considerable weight loss (17-19.5 percent) toward the 5th to 7th day after irradiation. However, head-shielded rats had recovered their initial weight /417 by the 25th day or even gained somewhat, while by the 30th day their weight was 112-114 percent of the initial level. At the same time, control rats and haunch-shielded rats even after 30 days had not completely regained their weight loss. A more detailed analysis of certain features in the clinical course of radiation sickness among the rats with shielding of parts of the body is given below.

In succeeding experiments we investigated more thoroughly the effect of abdominal shielding by blocks of various widths on the course of radiation reaction among rats exposed to gamma and 120 MeV proton irradiation in various dose ranges.

Study of the effects of abdominal shielding with blocks of various widths on the course and result of radiation reaction during total body proton

irradiation were conducted on 233 rats in two dose ranges: 800-1,050 rad and 1,100-1,300 rad, while with gamma radiation 274 rats were used, with doses of 930, 1,100 and 1,400 rad.

The pulse character of the proton flux and the fluctuations in its intensity during irradiation, as well as the complexity of the dosimetry, led to a definite scatter in radiation doses of animals in different groups. /419 For this reason we could not avoid combining into several groups those animals which received radiation doses varying by 100 to 200 rad. In use, the experimental gamma source afforded considerably greater stability and precision in radiation dose measurement. This permitted us to evaluate the influence of screens of various widths on the survival rate of rats exposed to various gamma-radiation doses with a higher degree of confidence than was possible for proton irradiation.

The results of the experiments, in terms of survival rate and average time-to-death among the rats that perished, are set forth in table 3. The table shows that shielding the abdomen with an organic glass block 6 cm wide during 800 to 1,050 rad proton irradiation raised the survival rate of the animals to 86.4 percent, as compared with 19.4 percent in the control group. A 4 cm screen had a less positive effect, but this difference is not statistically reliable. However, a 2 cm screen dropped the survival rate to 50 percent, a statistically reliable difference both from the controls and the groups with screens 4 and 6 cm wide.

The high effectiveness of the 6 cm block was retained in the 1,100 to 1,300 rad tests: 70 percent survival, as against 4 percent among the controls. The drop from 86.4 percent in the previous test to 70 in this one was not statistically reliable.

Total 930 rad gamma irradiation with abdominal screening (blocks 6, 4 and even 3 cm wide) gave a very high and practically identical survival rate, from 96.7-100 percent. When the width of the shield was reduced to 2 cm the survival rate dropped to 83.3 percent, but this result also testifies to the great effectiveness of abdominal shielding. The results of an experiment conducted with a small number of mice exposed to 1,100 rad confirmed the positive influence of abdominal shielding.

In the next experiment, which was accomplished using a large number of animals, the radiation dose was raised to 1,400 rad. At this dose all 12 control rats died during the first 4 days after irradiation. At the same time, a 4 cm block screening the abdomen produced 88.9 percent survival (16 rats out of 18), while shields 3 and 2 cm wide did not alter this result: in both cases 14 out of 17 survived (82.4 percent). Subsequently, during exposure to a gamma dose exceeding by more than one and a half times the minimum absolute lethal dose for rats (by our data $\sim$ 850 rad), abdominal /420 shielding with blocks 2-4 cm wide produced a very strong effect: the survival rate of these animals was 82.4-88.9 percent, while all controls perished.

Shielding the abdomen during total body irradiation not only raised the animals' survival rate, but also substantially altered the clinical course of

TABLE 3. EFFECT OF SHIELDING THE ABDOMEN WITH BLOCKS OF VARIOUS WIDTHS ON SURVIVAL RATE OF RATS.

Type of radiation and dose, rad		Width of shield, cm	No. of rats	No. of rats surviving	Survival rate		Average lifetime, in days, of rats who died (M ± m)
					% (M ± m)	Reliability of difference from radiation control	
Gamma rays	930	Radiation control	48	3	6.3±3.5	-	7.0±0.4
		6	30	30	100	-	-
		4	30	29	96.7±3.3	p<0.001	-
		3	36	35	97.2±2.8	p<0.001	-
		2	30	25	83.3±6.8	p<0.001	18.2±4.3
	1100	Radiation control	12	0	0	-	4.8±0.4
		4	12	12	100.0	-	-
		2	12	11	91.7±8.0	-	-
	1400	Radiation control	12	0	0	-	3.3±0.1
		4	18	16	88.9±7.4	-	18.0(2)
		3	17	14	82.4±9.2	-	18.0(3)
		2	17	14	82.4±9.2	-	16.3(2)
Protons	800-1050	Radiation control	72	14	19.4±4.7	-	9.1±0.6
		6	44	38	86.4±5.2	p<0.001	10.7±3.0
		4	36	28	77.8±6.9	p<0.001	8.8±2.3
		2	24	12	50.0±10.2	p<0.01	11.4±1.8
	1100-1300	Radiation control	27	1	3.7±3.5	-	6.6±1.0
		6	30	21	70.0±8.4	p<0.001	14.8±1.6

radiation sickness. The most typical symptoms of radiation sickness, as well as the ones most accessible to observation, manifested themselves in the rats more rarely and less strongly than in the controls. We studied the change in clinical phenomena of radiation injury during use of shielding in greater detail when making the proton experiments. It must be noted that such a typical symptom of early severe radiation injury to the intestines as diarrhea was, during proton irradiation in lethal or sublethal doses, much more clearly exhibited and was observed in a larger percentage of the control animals than during gamma irradiation. Shielding the abdomen with a screen 6 cm wide resulted in a statistically reliable reduction in the frequency of diarrhea. After 800 to 1,050 rad, 62 percent of the control group developed diarrhea within 2 to 3 days; the incidence remained at about the same level (59 percent) for the next 2 days and ceased only toward the end of the second week. The 6 cm shielding prevented all but 13.6 percent from developing diarrhea in the first 2 to 3 days, while 29.4 percent of the rats manifested this symptom with a 4 cm screen. For the two shield thicknesses the rates after 4 to 5 days were 6.8 and 9.1 percent, respectively. By the sixth day none of the rats exhibited this symptom.

At a higher proton dose (1,100 to 1,300 rad), 78 percent of the controls developed severe diarrhea in the first 2 or 3 days. Rats severely afflicted by diarrhea died within 4 or 5 days after irradiation. Rats protected by the shield manifested a statistically reliable reduction in diarrhea frequency (13-17 percent in the first 5 days) and fairly rapid cessation. The abdominal shield thus guarded the intestines against severe radiation injury and sharply reduced the early deaths that otherwise would have occurred.

Abdominal shielding also affected weight changes. While it did not prevent a loss during the first 3 to 5 days after irradiation, the percentage change in body-weight was nevertheless considerably reduced (fig. 2). After an 800-1,050 rad irradiation the loss was 7-8 percent in the shielded group and 11.7 to 13.8 percent in the controls. With an increase of 1,100-1,300 rad the losses were 7.6 and 10.6-12 percent, respectively. An even /422 greater difference was observed in the speed and degree of recovery of weight. None of the control rats who survived the 30 day observation period succeeded in regaining their weight, while those afforded shielding had returned to the initial level by the 15th or 20th day and had gained even more weight by the end of the observation period. The weight difference between control and experimental rats over the 30 day span is statistically reliable.

It is worthy of note that the majority of control rats who survived the 30 day observation period died in the course of the second month, while death occurred only in individual cases among the shielded rats following the observation period. However, a deeper evaluation of the reactive state of the rats 30 days and longer after irradiation is a matter for special investigation.

Weight losses following gamma irradiation followed much the same pattern as after proton exposure. Thus, as figure 3 shows, all groups exhibited a significant weight loss by the 3rd to 7th day after a 930 rad dose. In the control group the reduction was almost 20 percent, while rats protected by a 6 cm block experienced an 8.6 percent drop (17.6 percent with a 2 cm block). By the end of the 30 day observation period those control rats who survived

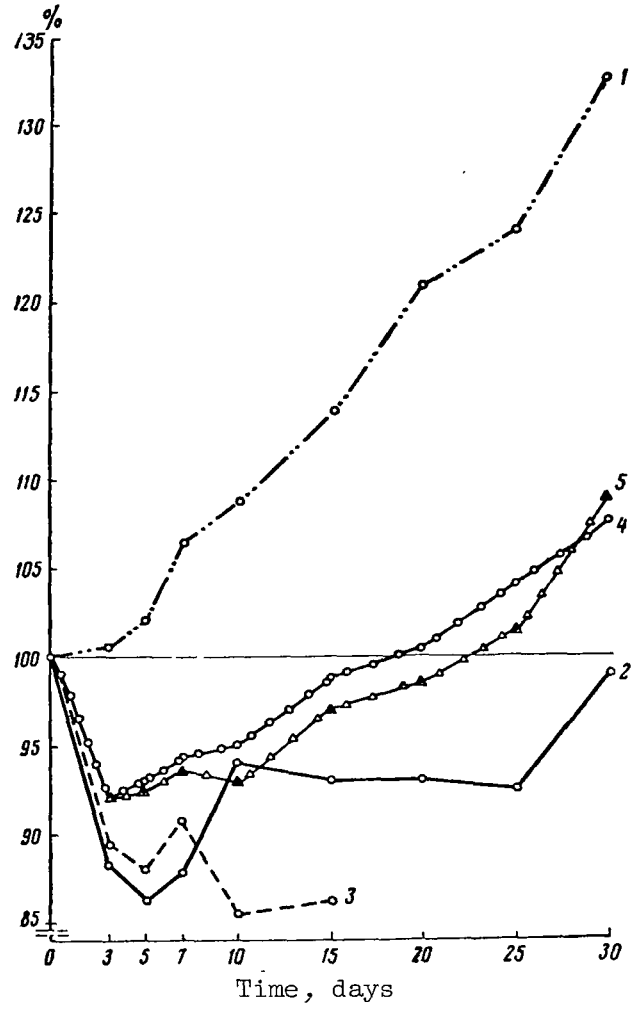

Figure 2. Graph of weight change in rats during proton irradiation. 1, Biological control; 2, radiation control (800-1,050 rad); 3, radiation control (1,100-1,300 rad); 4, abdominal shielding (800-1,050 rad); abdominal shielding (1,100-1,300 rad).

had come up to only 91.8 percent of their initial weight, while rats with 4 and 6 cm shields had returned to their prior weight by the middle of the period. With a 2 cm block the return was slower. All abdominally shielded groups had exceeded the initial weight level, by 12-19 percent, at the end of 30 days. With a 1,400 rad dose the shielded rats underwent an even sharper weight loss in the first days after irradiation: 21.3 percent with a 4 cm screen and 25.9 percent with a 2 cm screen. However, even at this dose, which effected the death of all controls within 3-4 days, the abdominally shielded rats had returned to their former weight by the 15th-20th day and exceeded it by 10-12 percent after 30 days.

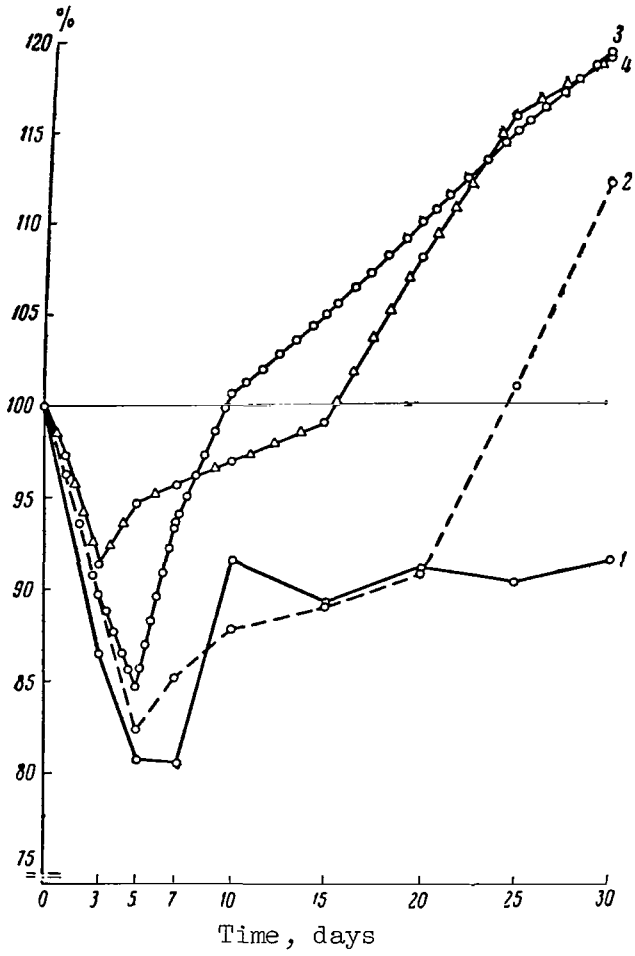

Figure 3. Weight changes in rats during gamma irradiation (930 rad). 1, Radiation control; 2, abdominal shielding (width: 2 cm); 3, abdominal shielding (4 cm); 4, abdominal shielding (6 cm).

Investigation of changes in leukocyte count in the peripheral blood showed that both types of radiation and both dosages produced definite leukopenia in both controls and abdominally shielded rats on the third day after irradiation; there were no significant differences from one group to the next. The leukocyte count was 7-14 percent of the initial number (fig. 4). At a radiation dose of 1,100-1,300 rad leukopenia was quite pronounced even on the 7th day after irradiation, while after a smaller dose (800-1,050 rad) the abdominally shielded animals exhibited a statistically reliable increase in leukocyte count /424 compared with the control group (35.8 as against 11.2 percent). After 15 and 30 days all groups in both dose ranges manifested a considerable restoration of leukocyte count, especially at the smaller radiation dose, although not a single group, even after 30 days, had completely recovered the initial level.

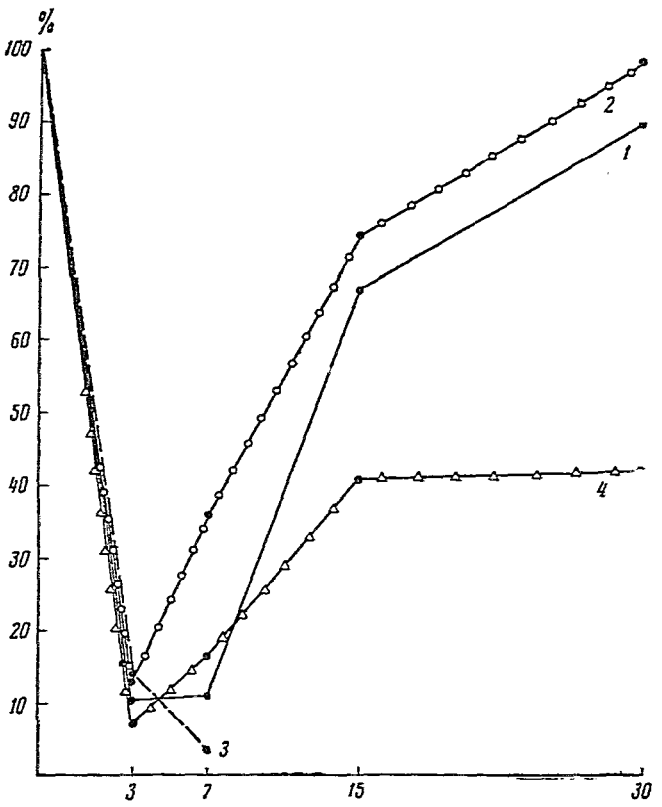

Figure 4. Changes in average number of leukocytes during proton irradiation.
1, Radiation control (800-1,050 rad);
2, abdominal shielding (800-1,050 rad);
3, radiation control (1,100-1,300 rad);
4, abdominal shielding (1,100-1,300 rad).

After a 930 rad gamma dose, as comparison of figures 4 and 5 will show, the pattern of change in leukocyte count was very like that described. /425 All groups experienced pronounced leukopenia by the third day, but its intensity was in inverse proportion to the width of the shielding block. Among controls the leukocyte count was only 10.6 percent of the initial number, while with a shield 2 cm wide it was 16.2 percent, 28 percent with a 4 cm block and 35.6 percent with a 6 cm block. By the 7th day the leukocyte count continued to drop among the control rats, while at the same time all groups of abdominally shielded rats began to regain the initial level, and by the 30th day the count had practically reached this level.

In preliminary experiments we investigated the influence of abdomi- /426 nal shielding on the course of radiation reaction in rats during combined action of protons and acceleration (or vibration) applied one day before and after irradiation. The animals underwent 10 G centrifugation for 30 min.

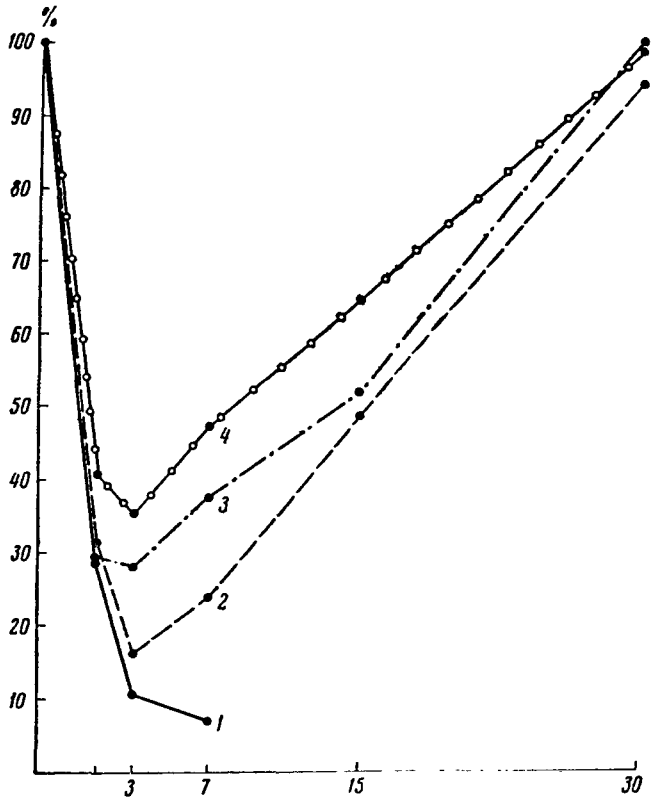

Figure 5. Changes in average number of leukocytes during 930 rad gamma irradiation. 1, Radiation control; 2, abdominal shielding (width: 2 cm); 3, abdominal shielding (4 cm); 4, abdominal shielding (6 cm).

Vibration at a frequency of 700 cps and amplitude of 0.005 mm lasted 1 hr, with a 10 G lead. The proton dose in these experiments was 750-1,100 rad in combination with centrifugation and 1,050-1,300 rad with vibration.

The data obtained permit us to asseverate that neither acceleration nor vibration under the given conditions substantially affected the way in which abdominal shielding affected the course of radiation reaction among rats. In these experiments the survival rate of abdominally shielded rats was 70-90 percent (table 4). Certain numerical differences in survival percentage were found between separate groups exposed to the same radiation-dose range, both with and without acceleration (or vibration), but these proved to be statistically unreliable. Just as with proton radiation alone, the abdominal shielding modified the clinical symptoms of radiation sickness, including a /427 sharp reduction in the frequency and severity of diarrhea.

TABLE 4. EFFECTIVENESS OF ABDOMINAL SCREENING DURING COMBINATION ACTION OF PROTON IRRADIATION, VIBRATION AND ACCELERATION.

Radiation dose range, rad	Type and sequence of action	No. of rats	No. of rats surviving	Survival rate, % (M ± m)
750-	Abdominal shielding plus irradiation	44	38	86.4±5.2
1,050 (1,100)	Acceleration plus abdominal shielding plus irradiation	32	25	78.1±7.3
	Abdominal shielding plus irradiation plus acceleration	20	18	90.0±6.7
1,100 (1,050)-	Abdominal shielding plus irradiation	30	21	70.0±8.4
1,300	Vibration plus abdominal shielding plus irradiation	21	13	61.9±10.6
	Abdominal shielding plus irradiation plus vibration	24	15	63.3±9.8

We discovered no outstanding peculiarities in the nature of changes in weight and total number of leukocytes among the control and shielded rats. The basic patterns of change in these parameters were preserved both with proton irradiation alone (over the same dose range) and with combined action.

We will now discuss the overall evaluation of the effect of shielding on the survival rate of animals during irradiation with lethal and sublethal doses. If we compare the effect of shielding for different dose rates by using the resulting survival rates, we can conclude that the effectiveness of shielding for 1,100-1,300 rad proton irradiation is 16.5 percent less than with an 800-1,050 rad dose. However, such a conclusion is not entirely true, because it does not take into account the survival rate of controls exposed to various doses. A more correct concept of the effectiveness of the method can be gained from the index of survival: the ratio of percent survival in the experimental group to the percent survival for the control group. For the 6 cm shield this ratio is 5.0 in the 800-1,050 rad range and 19.0 in the 1,100-1,300 rad range. Thus the effectiveness of the shield, judged by the index of survival, increases as the radiation dose increases. This conclusion contradicts the first one.

The question of the mechanism by which shielding various parts of the body exerts a positive effect on the course of radiation sickness during total body irradiation of animals is quite complex and is subject for independent investigation. In this report we will limit ourselves to a few remarks on this matter.

During irradiation of the animals with a directed beam of radiation a screen of sufficient thickness placed in front of some sector of the body absorbs the basic mass of incident rays and thus prevents irradiations of the shielded areas of tissue. Consequently, the total amount of radiation energy absorbed by the tissues of the entire body of the animal during total body radiation, with screening of any area whatever, will be less than in the control animal exposed to the same dose.

On the basis of the data contained in table 1 we can, with a tolerable degree of precision, determine the relative magnitude by which the absorbed dose is reduced. For example, when a shield 4 cm wide is used to screen the abdomen the absorbed dose drops approximately by a quarter (25 percent). Consequently, screening parts of the body is a method which permits us to /428 reduce the radiation dose through absorption of radiation energy by the material from which the block is made. However, can we explain the shielding effect only by a reduction in the absorbed radiation dose? Obviously not. During gamma irradiation of rats in a 1,400 rad dose, with abdominal shielding by a block 4 cm wide, the radiation is reduced by about 25 percent, i.e., to about 1,050 rad. During total body irradiation of rats without shielding this dose is absolutely lethal. However, in our experiments the use of a 4 cm shield during 1,400 rad irradiation prevented death in all but an insignificant number of rats. The overwhelming majority of animals--about 90 percent--lived. This example graphically shows that the effect of shielding is explained not so much by a reduction in the absorbed radiation dose as by preservation in the irradiation organism of sectors of uninjured tissues, located at the moment of irradiation behind the shield.

Some investigators (refs. 12 and 13) reckon that any biological tissue undamaged by radiation is in condition to undergo a less severe course of radiation reaction. This, evidently, is true only with relatively small injurious doses. At doses which are fatal to a preponderant number of animals, the most stable effect on the course of postradiative reaction is afforded by shielding the radiosensitive organs, primarily the bone marrow, spleen and small intestine (refs. 7, 8, 11 and 16-19). When sections of the upper part of the abdomen are screened, the greatest success in preventing injury is realized in the hematopoietic tissue (bone marrow and spleen) and small intestine.

Our experiments confirm earlier observations made by other authors that shielding at the time of total body gamma or X-ray irradiation of sectors of the body of animals exerts a substantial effect on the course of radiation sickness, reducing the severity of clinical symptoms and sharply cutting back the number of fatalities.

We have shown, first of all, that the same beneficial effect is realized when the radiation is in the form of 120 MeV protons.

The most pronounced positive effect on the course of radiation sickness and on the number of fatalities after total body irradiation in sublethal, lethal and superlethal doses is brought about by shielding various sectors of the upper half of the abdomen.

Neither acceleration nor vibration on the day before and the day after total body proton irradiation affected the nature of the effect which shielding the upper part of the abdomen has on radiation reaction.

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MORPHOLOGICAL CHANGES IN THE HEMATOPOIETIC ORGANS OF MICE IRRADIATED WITH HIGH-ENERGY PROTONS

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V. V. Antipov and P. P. Saksonov

One of the spaceflight factors that adversely affects the organism /430
is cosmic rays, some 80 percent of which consist of protons (refs. 4 and 8).

It was not until recently that reports began to appear on the biological effect of high-energy protons. Some authors (refs. 1, 10 and 13) found that the relative biological effectiveness (RBE) of protons is lower than that of gamma rays.

This article describes the results of a comparative study of the dynamics of pathomorphological changes in the hematopoietic organs of adult male mice of the C-57 line after exposure to protons and gamma rays. Three series of experiments involving 30 animals in each were performed.

The first group of animals was irradiated once with 830 rad of protons by a collimated beam with an energy of 400-600 rad/min in the 6 meter synchrocyclotron of the Combined Institute of Nuclear Research.

In the second series, the animals were irradiated with 650 r of gamma rays from Co^{60} at a dose rate of 273 r/min.

In the third series, the animals were not irradiated and they served as control.

The animals were sacrificed with ether fumes 3, 7, 15, 30 and 60 days following exposure after they had been systematically observed, with note taken of changes in weight, condition and behavior. Bone marrow from the femur, spleen and thymus were fixed in Carnoy's fluid and embedded in celloidin for microscopic examination. Decalcification of the femur to study the bone marrow was carried out by Willenson's method in 10 percent nitric acid solution in 5 percent formalin. All organs were stained with hematoxylin-eosin to detect /431
ribonucleoproteins in the spleen and thymus. The sections were treated with methyl green pyronine by Brachet's method with control of ribonuclease. Cytological control was effected on spleen and bone marrow impressions and smears stained by Pappenheim's and Brachet's methods.

Some of the animals developed diarrhea between the 3rd and 7th days after irradiation with protons or gamma rays. The mice became untidy and their fur was disheveled and lusterless. These phenomena disappeared about the 15th day. There was a patchy loss of hair between the 20th and 30th days, and the hairless portions of skin became covered with dry crusts between the 45th and 60th days. At this time areas of gray hairs 0.3 to 0.5-0.7 cm in size appeared on the back, less commonly on the sides of the animals.

The animals died between the 9th and 12th days after exposure. The animals that died of radiation lesions generally had large hemorrhages under the skin and in the lungs and intestine. In the animals that were sacrificed, hemorrhages were found only in the lungs on the 3rd and 7th days after exposure.

The weight coefficients of spleen and thymus were determined when the animals were dissected. The results are summarized in the table.

It is evident from the table that there was a statistically significant decrease in the weight coefficient of the spleen ($p < 0.001$) and thymus ($p < 0.001$) in the animals irradiated with protons, as compared with the intact mice, as early as the 3rd day. The coefficient tended to increase on the 7th day. /432 By the 15th day the weight coefficient of the spleen increased significantly ($p < 0.05$); it decreased about the 30th day but increased again about the 60th day. The weight coefficient of the thymus was restored somewhat more slowly, although it did not exceed that of the thymus in the control animals ($p = 0.25$).

The differences between the weight coefficients of the spleen and thymus of the animals irradiated with protons and gamma rays were statistically insignificant and thus ignored in determining the RBE of the protons.

The histomorphological changes in the hematopoietic organs of the irradiated animals were as follows.

In the spleen of the animals irradiated with protons, the follicles shrank considerably about the 3rd day and the number of lymphocytes in the organ decreased sharply. There were no centers of multiplication. Some of the pyroninophilic cells surviving in small aggregations resembled plasma cells, with much fewer mitotic figures than in the control. Foci of myelopoiesis were lacking in the pulp. The number of megakaryocytes decreased and the venous sinuses were dilated.

About the 7th day after irradiation with protons, the lymphocyte content of the follicles decreased even more, but the number of pyroninophilic cells increased. The latter were scattered throughout the follicles, with more mitotic figures than on the 3rd day. Erythroblasts and pyroninophilic cells with mitotic figures appeared in the pulp under the capsule.

On the 15th day after irradiation with protons, the follicles of the spleen were bigger than usual. They consisted of large round cells with a light nucleus, pyroninophilic cytoplasm and numerous mitotic figures. The pulp was filled with pyroninophilic cells, megakaryocytes and cells of the myeloid series arranged in polymorphic cell strands. Among the erythrocytes in the sinuses were many lymphoid, myeloid and even immature pyroninophilic cells.

CHANGE IN WEIGHT COEFFICIENTS OF SPLEEN AND THYMUS OF MICE IRRADIATED WITH PROTONS AND GAMMA RAYS (M±m)

Day dissected (after irradiation)	Spleen			Thymus		
	Protons 830 rad	Gamma rays 650 r	Control	Protons 830 rad	Gamma rays 650 r	Control
3rd day	0.149± ±0.008	0.132± ±0.001	0.411± ±0.030	0.067±0.008	0.056±0.003	0.219±0.009
7th day	0.111± ±0.021	0.143± ±0.007	0.382± ±0.015	0.103±0.010	0.073±0.016	0.261±0.035
15th day	0.555± ±0.107	0.641± ±0.063	0.453± ±0.042	0.160±0.054	0.061±0.013	0.227±0.008
30th day	0.450± ±0.013	0.378± ±0.014	0.350± ±0.013	0.208±0.030	0.153±0.027	0.152±0.033
60th day	0.614± ±0.079	0.732± ±0.081	0.358± ±0.038	0.169±0.015	0.125±0.007	0.124±0.014

On the 30th day after irradiation, the follicles were ordinary in size with fairly distinct boundaries and they contained a substantial number of mature lymphocytes. Mitotic figures and pyroninophilic cells were found only in the centers of multiplication, which were well formed by that time. The pulp had fewer pyroninophilic cells than before and mitotic activity had diminished. Foci of myelopoiesis were noted only under the capsule and around the trabeculae. They consisted mainly of erythrocytes.

By the 60th day, the follicles of the spleen in the animals irradiated /433 with protons, unlike the control, were less distinct. The centers of multiplication were somewhat enlarged and hyperplasia of the foci of myelopoiesis in the pulp was more extensive than in the nonirradiated animals. Pyroninophilic cells were present. In some animals, the weight of the spleen was much greater than that of the control. In such cases, the pulp had necrotic foci saturated with blood and fibrin. There was virtually no cell reaction around these foci.

Destruction of the spleen and suppression of mitosis were pronounced between the 3rd and 7th days in the animals irradiated with gamma rays. By the 15th day, pyroninophilic cells and mitotic figures were more numerous in the pulp and erythroblasts and myeloblasts less numerous than in the mice irradiated with protons. Polymorphic cell strands were not yet in evidence. Thrombi were found in the venous sinus of the spleen in some of the animals. Maturation of myelopoiesis in the pulp (increased content of the more mature cell forms in the foci of myelopoiesis--erythroblasts and myeloblasts--formation of polymorphic cell strands instead of solid fields consisting of pyroninophilic undifferentiated mitotically active cells) was observed only about the 30th day. Hyperplasia of

the foci of myelopoiesis remained extensive even on the 60th day. Necrotic foci were found more frequently at this time in the pulp than after irradiation with protons. Organized thrombi could be seen in the sinuses.

The start of restoration of the follicles, as in the animals irradiated with protons, was noticeable on the 7th day, but the process was slower and did not end until the 60th day. As in the animals irradiated with protons, the follicles of the spleen were loose with enlarged centers of multiplication. Beyond the latter were mitotic figures in the gamma-irradiated animals.

In the animals sacrificed on the 3rd day after irradiation with protons, there were relatively few mitotically dividing cells in the thymus and lymphocytes had disappeared from the cortical substance. By the 7th day, ribonucleoproteins accumulated in many reticular cells of the cortical substance, while more lymphocytes were formed in the stroma. These lymphocytes were rather large with loose nuclei and a distinct rim of pyroninophilic cytoplasm. The cortical substance was somewhat enlarged. By the 15th day, the cortical substance, still enlarged, had many mature lymphocytes and mitotically dividing pyroninophilic cells. On the 30th day, the structure of the thymus was normal.

On the 3rd day after irradiation with gamma rays, there was rather marked destruction of the reticular stroma of the cortical substance. Restoration of the gland was rather slow, and by the 7th day only the relatively narrow /434 subcapsular zone of the cortical substance contained lymphocytes and reticular cells with pyroninophilic cytoplasm. Mitotically dividing cells were less common than in the animals irradiated with protons. Hyperplasia of the cortical substance was noted on the 30th day and restoration on the 60th day.

Three days after irradiation with protons, the bone marrow of the animals contained only small islands of hematopoietic cells. On the 7th day, near the swollen cells of the endosteum and around the bony trabeculae and blood vessels could be seen masses of large round cells with dark chromatin-saturated nuclei containing a few erythroblasts and solitary megakaryocytes. About the 15th day, the entire stroma of the bone marrow was filled with hematopoietic elements--erythroblasts and masses of megakaryocytes. Mature forms of the myeloid series were virtually nonexistent. About the 30th day, there were fewer immature cell elements in the stroma of the bone marrow than before. Segmentonuclear leukocytes were found more frequently, and the bone marrow had regained its normal structure.

About the 3rd day, hematopoietic elements were absent in the bone marrow of the gamma-irradiated animals. The stroma was edematous and here and there saturated with blood. By the 7th day, a few immature, round, undifferentiated cells with a dark nucleus appeared in the destroyed bone marrow. The stroma did not become filled with hematopoietic elements until the 30th day. The bone marrow regained its normal structure about the 60th day when segmentonuclear granulocytes appeared and the normal ratio of the cell forms was more or less restored.

Observations of the animals and a comparative study of the spleen, thymus and bone marrow following exposure to protons and gamma rays reveals that the

ensuing changes were similar. The weight coefficients of the spleen and thymus changed identically at the various periods of the investigation.

During the first few days, the follicles of the spleen shrank and the foci of myelopoiesis disappeared from the pulp. In the thymus, the cortical substance lost its lymphocytes, while the reticular stroma was clearly destroyed in the bone marrow.

It will be noted that all changes were less pronounced after irradiation with 830 rad of protons than after irradiation with 650 r of gamma rays. For example, the size of the follicles in the spleen return to normal by the 15th day; their normal morphological structure, by the 30th day. Foci of myelopoiesis in the pulp appeared sooner in the animals irradiated with protons and became hyperplastic by the 15th day, achieving considerable maturity; by /435 the 30th day they had regained their normal appearance. After gamma irradiation the foci of myelopoiesis matured more slowly and remained hyperplastic until the 30th day, but the morphological structure of the spleen did not return to normal until the 60th day.

The spleen was not fully restored by the 60th day in any of the animals, whether exposed to protons or gamma rays. Necrotic foci were found in the pulp. They were more numerous and larger in the gamma-irradiated animals whose splenic sinus also contained organized thrombi.

The structure of the thymus was also restored more rapidly in the animals irradiated with protons. Hyperplasia of the cortical substance was noted on the 7th and 15th days. The gland was fully restored about the 30th day. In the animals irradiated with protons, hyperplasia of the cortical substance of the thymus was noted on the 15th and 30th days; restoration of the morphological structure, on the 60th day.

Although less marked than after gamma irradiation, the initial destruction of bone marrow in the proton-irradiated animals did not show clear signs of restoration until the 7th day. On the 15th day, the entire stroma was filled with cell elements. The bone marrow matured and the normal ratio of the cell forms was restored about the 30th day.

In the gamma-irradiated animals, the signs of restoration in the bone marrow were barely perceptible on the 7th day, but clearly evident on the 15th day (by this time two-thirds of the stroma of the femoral bone marrow was filled with cell elements). The stroma was not filled until the 30th day, while the normal morphological structure of the bone marrow was restored by the 60th day.

Many authors made comparative studies on the biological effects of various kinds of ionizing radiation. They found that the effects of radium and roentgen rays are alike and that they result in the death of lymphoid elements and destruction of the spleen (ref. 15). L. M. Gorovits' findings (ref. 5) on radium rays were similar. A comparison of the effects of neutrons and gamma rays failed to turn up any morphological differences in the morphological reactions of the animals (ref. 6). I. V. Toroptsev and N. V. Sokolova (ref. 9) observed

a typical picture of radiation sickness induced by rays generated by a betatron with an energy of 10 MeV. However, the degree of injury, i.e., the biological effectiveness of different kinds of penetrating radiation, varies (refs. 2, 3, 7 and 14) with their physical nature (refs. 16 and 17).

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RESISTANCE OF RATS TO SEVERE OXYGEN DEFICIENCY DURING RADIATION SICKNESS

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Ionizing radiation and oxygen deficiency are among the most dangerous /437 pathogenic factors that endanger manned spaceflights. An oxygen deficiency may arise under a variety of emergency circumstances, e.g., puncture of the hermetic seal of the cabin or spacesuit or breakdown of the air regenerating system. It may also result from circulatory disorders caused by acceleration.

An oxygen deficiency is not usually an isolated factor in space, but may well be combined with such factors as ionizing radiation. The two may occur simultaneously or successively. Moreover, sensitivity to one may be specifically changed by the preceding factor.

The simultaneous effect of hypoxia combined with ionizing radiation has been studied in considerable detail in a wide range of objects. However, much less attention has been accorded to their successive effect, specifically the reaction to an oxygen deficiency by animals suffering from radiation sickness. Yet the question of whether sensitivity to hypoxia changes after irradiation has obvious practical as well as theoretical significance, because it may throw some additional light on the mechanism of action of ionizing radiation on oxidative metabolism in tissues.

There is no consensus in the literature on the nature of the effect /438 of ionizing radiation on the oxidative systems of animal tissues. Most investigators believe that radiation cannot injure the tissue oxidative systems, and that the slight changes that occur should not be regarded as an important factor in the development of radiation sickness (ref. 3). This view is largely based on negative evidence derived from in vitro studies of the oxidative enzymatic systems in the tissues of irradiated animals.

The contrary view, which is supported by some facts, is that inhibition of the function of the oxidative systems is one of the primary or at least early effects of radiation. The intensity of the oxidative processes was found to be inhibited in the course of an investigation of the oxygen content of rabbit brain tissue following exposure to lethal doses of radiation (ref. 8). There are some data which show that the main element in the mechanism that triggers radiation injury is specific damage to the iron-containing components of the oxidative enzymatic systems (ref. 7). Other authors (ref. 1) discovered that a major factor in the biochemical action of radiation is the dissociation of oxidation and phosphorylation, which has the same final effect as injury to the

respiratory systems, i.e., lowering of the level of the macroergic phosphorus compounds, or disruption of the energy balance of the cell.

Since ionizing radiation causes apparent or latent injury to tissue oxidative systems, we might expect that it would also sharply decrease the resistance of irradiated animals to hypoxia. The published references on the subject are fairly contradictory. For example, increased resistance was noted in mice irradiated with 325 and 700 r (ref. 15). V. A. Konstantinov (ref. 5) found that resistance to hypoxia increased in mice 1-2 days before they died as a result of irradiation with 1,000 r. The survival rate of rats kept for 4 hours in a pressure chamber at an "altitude" of about 8,000 m or more was found to increase considerably 1-3 days after irradiation with 500-600 r (ref. 12). The effect was much less pronounced at a lower "altitude." The authors believe that the increased survival rate resulted mainly from starvation, because at that time the irradiated animals were suffering from severe anorexia.

The same authors showed in a special series of experiments that rats fasted for 72 hours are almost as resistant to hypoxia as animals 72 hours after irradiation. However, they offer no explanation for the increase in resistance to hypoxia 24 hours after irradiation. The same study revealed that the survival rate of irradiated and control rats is the same in the case of autoasphyxia in airtight vessels. On the other hand, Manoylov (ref. 6) states /439 that irradiated mice are more sensitive to hypoxia than normal animals.

These findings do not readily lend themselves to comparison, because the experiments involved different animals, rates and intervals after irradiation. Moreover, the nature of the hypoxic agent applied varied in each of these investigations.

We set out to determine whether rat resistance to acute hypoxia changes in the course of acute radiation sickness. White male rats of the Wistar strain weighing 200-250 g were subjected to whole-body X-irradiation with 750 r. This dose caused acute radiation sickness manifested 3 days later in marked gastrointestinal disturbances. About 80 hours after exposure most of the animals began to die, and by the end of the 4th day only about 30 percent of the rats remained alive.

Immediately after exposure and 3, 6, 12, 24, 48, 72 and 96 hours later, groups of rats were "elevated" in a pressure chamber along with 2 or 3 control animals. The "elevation" to a negative pressure of 140 mm Hg took 5 minutes with 5 half-minute stops. When a pressure of 140 mm Hg was reached, the "elevation" was halted and the survival time of the animals at this "altitude" was recorded. The rats were regarded as dead as soon as the respiratory movements ceased. The survival time was recorded accurately to 15 sec. A total of 156 control and 178 irradiated animals were investigated in this series of experiments (15-28 animals in each of the post-exposure periods studied).

The data were used to calculate the mean lifespan of the rats at a pressure of 140 mm Hg. The results of the experiments were also processed by the method of probit analysis, which made it possible to calculate another and more significant value--the time of death of 50 percent of the animals.

It is evident from figure 1 that there was no significant difference in tolerance of acute hypoxia between the control and irradiated animals immediately after exposure and 3 hours later. But 6 hours later the lifespan at 140 mm Hg increased. It became longer 12, 24 and 48 hours after irradiation. The most substantial increase in resistance to acute hypoxia occurred after 72 hours, /440 i.e., at a time when the symptoms of radiation sickness were quite pronounced. At the last time interval investigated, when only about 30 percent of the rats were alive, their resistance to hypoxia was less than at the earlier interval (72 hours), but it was still higher than in the controls.

We conjectured that a possible cause of the increase in resistance to hypoxia in irradiated rats is the general reduction in intensity of the metabolic processes, since resistance to hypoxia is known to rise as the intensity of the metabolic processes falls, e.g., in hypothermia (ref. 11). The intensity of gas exchange and body temperature may serve as indices of the intensity of the metabolic processes.

As in the case of the reaction of irradiated animals to hypoxia, there is no consensus in the literature on the nature of the changes in gas exchange during acute radiation sickness. Some authors (refs. 9 and 10) found that the rate of O_2 consumption increased after irradiation, while others (refs. 14 and 15) failed to note any significant changes except when the animals had fasted, and the rate of O_2 consumption distinctly decreased. Other authors (ref. 3) observed a gradual decrease in gas exchange in mice irradiated with 600 r.

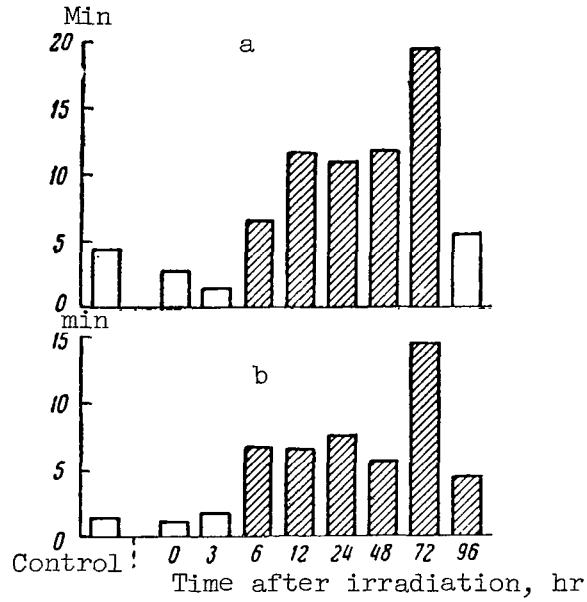

Figure 1. Mean lifespan in pressure chamber (a) and death of 50 percent of rats (b). Crosshatched columns correspond to values that differ in statistically significant fashion from control ($P < 0.05$).

In the terminal stage of radiation sickness, shortly before the animals died, the intensity of gas exchange decreased by about 50 percent. Following irradiation of rats with more than 400 r, gas exchange measured in the adaptation phase did not change the first day after exposure. However, it subsequently began to drop, reaching a minimum on the third day after exposure (ref. 3).

We systematically measured the rectal temperature and rate of oxygen consumption in rats irradiated with 750 r. No clear-cut temperature abnormalities were detected during the first two days of radiation sickness (fig. 2). On the third day after irradiation, the rectal temperature dropped by 3.9° on the average; on the fourth day, by 3.2°. /441

The rate of oxygen consumption was determined by the ordinary chamber method, with the air analyzed in a Haldane apparatus. Gas exchange was measured daily three days before irradiation and after irradiation at the same intervals as those when the survival rate was determined in the pressure chamber (i.e., 0, 3, 6, 12, 24, 48, 72 and 96 hours). At each of these times gas exchange was measured in 10-12 animals.

It is evident from figure 2 that oxygen consumption per unit of animal weight during radiation sickness changed very little. A significant difference in the intensity of gas exchange as compared with the control was noted only 72 hours after irradiation (decrease of 9 percent). Even in the terminal stage of acute radiation sickness, 96 hours after irradiation, there was no significant decrease in the intensity of gas exchange.

Thus, the increase in resistance to acute hypoxia noted in rats starting 6 hours after irradiation is not caused by a decrease in the intensity of the metabolic processes of the irradiated animals. This phenomenon is responsible, but only in part, for the higher survival rate 72 hours after irradiation. /442

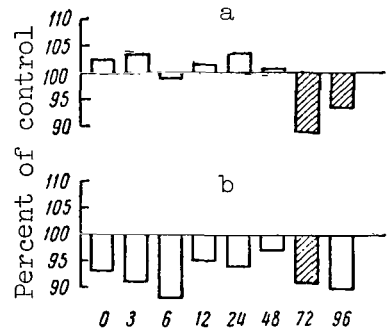

Figure 2. Rectal temperature (a) and oxygen consumption in ml/g·hour (b) during radiation sickness (values and given in percentages of mean control). Crosshatched columns correspond to values that are statistically different from control ($P < 0.05$).

We then conjectured that the increase in resistance to hypoxia may be caused not so much by the low original level of the metabolic processes after irradiation as by the quicker or deeper lowering of the level when the animals are exposed to hypoxic conditions, due to possible disruption of thermoregulation. This conjecture was based in part on the fact (ref. 2) that on cooling the drop in body temperature is quicker and deeper in irradiated animals than in the control.

In order to confirm this view, we performed a series of experiments in which the rate of oxygen consumption was determined during the time the animals were actually exposed to hypoxic conditions (ref. 4). Accordingly, oxygen consumption was determined in control animals and in animals 12 and 72 hours after irradiation, while breathing atmospheric air in a special gas exchange chamber. The air in the chamber was then replaced in 1 to 1-1/2 min with a gas mixture containing 7-7.8 percent oxygen, corresponding to a pressure of about 250-280 mm Hg, i.e., to an altitude of approximately 8,000 m.

The results of determining gas exchange by this method are similar to those in the preceding series of experiments. Oxygen consumption 12 hours after irradiation was at the normal level, but was lower after 72 hours. The extent of the decrease in oxygen consumption under hypoxic conditions (fig. 3) in the nonirradiated rats and in the irradiated rats 12 hours after exposure was the same (28 and 25 percent, respectively). But 72 hours after exposure the rate of oxygen consumption during exposure was 45 percent below the original level. Thus, the absolute level of gas exchange under these conditions was quite low.

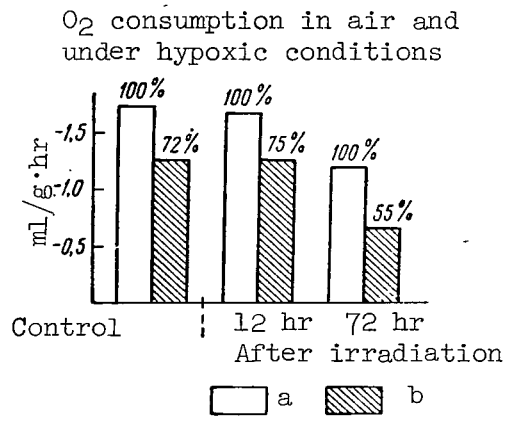

Figure 3. Oxygen consumption (in ml/g.hour) in air (a) and under hypoxic conditions (b, 7-7.8 percent O_2). Figures over columns indicate what percentage amount of O_2 consumed under hypoxic conditions is of amount consumed in air by corresponding group of animals.

It is clear from the foregoing that during acute radiation sickness, within 6 hours of irradiation, rat resistance to acute hypoxia increases. The increase persists until the terminal stage of radiation sickness, the peak of resistance occurring 72 hours after exposure. Analysis of the indices of gas exchange in the irradiated animals, under both normal atmospheric and hypoxic conditions, indicates that the mechanism governing the increase in resistance to hypoxia is not the same at the various stages of radiation sickness.

The increase in resistance 72 hours after exposure seems to be due to/443 the decrease in intensity of the metabolic processes in the sick organism, along with slight hypothermia probably resulting from starvation, intestinal disturbances, impairment of blood circulation, anemia and other abnormalities characteristic of this stage of radiation sickness. It may also be due to the fact that as a consequence of disrupted thermoregulatory and possibly some other factors at work in hypoxia, the intensity of gas exchange in rats with radiation sickness diminishes very sharply at this stage.

Resistance to hypoxia decreases 96 hours after irradiation below that occurring 72 hours later, and it approaches the level found in the control animals, although, judging by the low rectal temperature, the intensity of the metabolic processes continues to be somewhat low. This seems to be due to the impairment of all vital functions, which is characteristic of the terminal stage of radiation sickness and to a weakening of the organism and general decrease in resistance.

Concerning the increase in resistance to acute hypoxia during the earlier stages of radiation sickness, it obviously cannot be attributed to the same factors. It may well be caused by increased resistance in the phase of adaptation to such a powerful stressor agent as ionizing radiation. Further study is needed to elucidate the nature of the factors responsible for the increase in resistance to acute hypoxia during the early stages of radiation sickness.

At any rate, our data on the increase in resistance to the acute oxygen deficiency observed from the first few hours after irradiation until the terminal stage of acute radiation sickness, as well as the comparatively constant rate of gas exchange and body temperature (an indication of the absence of significant changes in the intensity of the metabolic processes in the irradiated organism), provide no grounds for believing that ionizing radiation in the doses used injures the tissue oxidative systems.

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NEW WAYS OF STUDYING CHEMICAL PROTECTION AGAINST GENETIC CHANGES

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In analyzing data obtained from experiments on lysogenic bacteria /445
carried aloft at the time of the first spaceflights in ships of the "Vostok" type, it was established that on the orbits during which the historic flights of Yu. A. Gagarin and G. S. Titov were accomplished, those conditions were absent which could substantially affect the phage-producing activity of Escherichia coli K-12 (λ) (refs. 1-3).

However, as orbital flights were lengthened, a definite influence of space factors on productive reactions in lysogenic cultures was discovered in the form of a small but (statistically) reliable increase in the level of induced phage manufacture in comparison with the amount of spontaneous phage formation in control specimens (refs. 4 and 12).

According to modern concepts, spontaneous and induced phage formation are related to the presence of a prophage resulting from integration of phage DNA fragments with a bacterial chromosome during interaction of mild phages with microbe cells. The prophage as carrier of the genetic determinant of the phage is closely linked to the bacterial chromosome and behaves like a hereditary characteristic belonging to the lysogenic bacteria. However, the acquisition of such heredity by the lysogenic bacteria leads to their genetical instability with a tendency toward pathological changes and--as was shown in references 7-9--toward a sharp increase in the susceptibility of the genetic apparatus of the microbe to the effects of ionizing radiation.

The search for prophylactic agents against genetic damage becomes /446
especially pressing in connection with the future achievement of more remote orbits and with the increase of spaceflight duration, when the genetic dangers will be of vital importance (ref. 5).

In preliminary experiments testing this model of chemical bonds with confirmed antiradiation properties (cysteine, cystamine), it was shown that the protection against radiative induction given by these compounds manifests itself as an abrupt drop in the quantity of induced phage particles after irradiation of a lysogenic strain of E. coli K-12 (λ) by X-rays.

At the same time, contact between the culture and a preparation without antiradiation properties (e.g., urethane) did not result in any observed reduction in induced effect of X-rays. In other words, the number of induced

phage particles after treatment of the culture by urethane and exposure to X-rays was the same as in specimens irradiated without the preparation (ref. 6).

It thus became clear that chemical compounds with antiradiation properties are able to some degree or other to block one of the stages by which genetic reaction occurs, viz., X-ray-induced transformation of the prophage into a mature phage.

These data permit us to undertake a much broader investigation of chemical compounds on this model, for the purpose of finding not only effective antimutagenic, but also effective mutagenic substances, possessing protective properties (against genetic damage) or, on the contrary, potentiating induced phage formation. In either case the selection and study of such substances has not only a theoretical, but indubitably also a practical significance.

The task of the present study is to investigate chemical compounds of the aminothiol group, as well as certain analogs of pyrimidine bases from the standpoint of their prophylactic properties against genetic damage caused by action of X-rays on lysogenic bacteria.

The table lists chemical substances of the aminothiol group and pyridine-base analogs tested for their capacity to inhibit the development of infectious phage from prophage after induction of E. coli K-12 (λ) by X-rays. These preparations were synthesized under the supervision of M. N. Volkov.

All preparations were subjected to preliminary testing for their bactericidic and bacteriostatic effects. In succeeding experiments the preparations were used in doses which did not occasion a toxic effect on the lysogenic strain.

For all chemical compounds used, this nontoxic dose corresponded to /447
a concentration of 0.05 percent.

The methodology was as follows: a 5 hr broth culture of E. coli K-12 (λ) seeded in a 1:10 physiological medium; this solution was divided into two equal parts, and one or another of the tested preparations was added to one of the cultures in the indicated concentration.

Experimental and control (without the preparation) specimens were incubated for 30 min at 37° and then simultaneously exposed, in a special container, to a predetermined dose (15,000 r) of X-radiation.

After irradiation we made appropriate dilutions of specimens of the culture and seeded them by the agar-layer method together with an indicator strain of E. coli C-85.

The test results were evaluated by comparing the quantity of induced phage particles in the irradiated specimens of E. coli K-12 (λ) treated with a preparation and in the irradiated, untreated controls (coefficient K).

From the table it is apparent that almost all preparations in the aminothiol group reduce the level of induced phage production during X-irradiation

EFFECT OF CHEMICAL PREPARATIONS ON PHAGE-FORMATION ACTIVITY IN CULTURE OF E. COLI K-12 (λ) AFTER 15,000 r X-IRRADIATION.

Preparation and its formula	K*
2-mercaptopropylamine hydrochloride	119.0
2-(γ-aminopropyl) disulfide dihydrobromide	76.3
Sodium diethyldithiocarbamate	72.1
Ammonium dithiocarbamate	70.1
Azacytidine	54.4
2-mercaptoethylamine hydrobromide	46.4
Sodium dimethylaminodithiocarbamate	39.2
β-aminoethyl disulfide dihydroiodide	33.8
3-mercaptopropylamine hydrochloride	14.7
Disulfide 2-mercaptoethylamine chlorhydrate	12.2
2-mercaptoethylamine hydroiodide	6.4
2-mercaptoethylamine hydrochloride	6.3
2-mercapto-4,6-dimethylpyrimidine	2.0
3-β-aminoethylisothiuronium hydrobromide	1.2

*K = R/P, where R is the quantity of induced phage particles after irradiation of E. coli K-12 (λ) without any preparation (control) and P is the quantity of induced phage particles after irradiation with a preparation (experiment).

of the lysogenic culture. It is notable that β-mercaptopropylamine proved /448 to be the chemical compound most effective in preventing prophage.

The amount of induced phage in the test specimens was 119 times less than the number of phage particles in the control culture.

Other compounds which had strong inhibitory effects on induced phage formation were 2-(γ-aminopropyl) disulfide dihydrobromide, sodium of diethyldithiocarbamate, and ammonium dithiocarbamate. With these the reduction was 70.1 to 76.3 times.

A less pronounced, but still distinct effect accompanied the use of β-aminoethyl disulfide dihydroiodide and the tested salts of β-mercaptoethylamine. However, aminoethylisothiuronium hydrobromide did not inhibit phage formation in the lysogenic culture, because the insignificant (1.2 times) reduction by comparison with the control specimens was statistically unreliable.

Analysis of the data obtained indicates the existence of a definite connection between the chemical structure of the test preparations and their capacity to inhibit development of infectious phages and thereby protect the lysogenic bacteria from the genetic effects of ionizing radiation.

The results of investigating the radioprotective action of familiar chemical compounds shows that replacement of the hydrobromide by the

hydrochloride of β-mercaptoethylamine considerably reduces the prophylactic properties of the preparation. In fact, the hydrochloride of this compound is a less effective agent than β-mercaptoethylamine hydrobromide against the genetic damage of E. coli K-12 (λ). The corresponding K values are 6.3 and 46.4 .

This fact is evidence that the antigenetic action of β-mercaptoethylamine preparations is determined not only by its basic, but also by its acid residues.

We therefore suggest the possibility of obtaining still more effective β-mercaptoethylamine preparations by forming their salts with other acids.

In addition, the results of investigating various substances indicate a definite dependence of the protective action of the preparations on their chemical structure. Thus, comparison of antigenetic effect of the hydrochloride salts of 2-mercaptopropylamine with those of 3-mercaptopropylamine in which first the hydrosulfide group occupies a more extreme position than in the former shows the latter to possess considerably less effectiveness. The K coefficients are 14.7 for the latter and 119 for the former.

The absence of antigenetic action in 3-β-aminoethylisothiuronium /449
hydrobromide is of great interest, although the contrary is reported in numerous papers on its effectiveness in various animals, where it reduced the death rate after a lethal dose by 70 to 100 percent (refs. 10-16).

This circumstance has special interest, because it opens new perspectives in the field of comparative investigation of the connection between the chemical structure of preparations and their capacity to guard the organism against severe radiation injury on the one hand and genetic sequelae on the other.

Recently quite effective results, surpassing those of other preparations, were given by "phinam," which possesses considerable capacity to ward off genetic damage and is at the same time less toxic.

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SIGNIFICANCE OF THE PROCESS OF POSTRADIATION REGENERATION OF GENETIC STRUCTURES FOR THE RADIOSENSITIVITY OF CELLS

COMMUNICATION 1. QUANTITATIVE LAWS OF POSTRADIATION RESTORATION OF YEAST CELLS

V. S. Barsukov, O. V. Malinovskiy and N. M. Mityushova

One of the important--and unavoidable--factors of spaceflight is /451
ionizing radiation. The passage of a spacecraft through the radiation belts of Earth, solar flares and also the use of nuclear power devices as engines can create a serious threat to the safety of the cosmonaut and other living things on board.

Under terrestrial conditions, when there is radiation danger we usually have recourse to tried and true physical means of protection: we increase the distance of the working area from the radiation source, or make a shield from material with a high coefficient of damping. Any scientifically established reduction in the maximum permissible dose for a comparatively small number of people working with an external radiation source can always be implemented by an engineering solution of the shielding problem.

Under spaceflight conditions, adequate physical protection cannot always be provided, owing to restrictions on the dimensions and weight of the cabin. Since we are far from being able to predict dose increases, the problem of providing radiation safety in spaceflights is made more complicated.

For this reason one of the important tasks of radiobiology is to develop ways of substantially reducing the harmful effects of ionizing radiation by treating the organism to be irradiated, i.e., the development of principles of biological protection.

As we know, the deaths caused by minimum lethal doses of the commonest types of radiation with a small linear ionization density are largely /452
the result of massive cell damage to the radiosensitive organs. These cells as a rule lose their reproduction viability as a result of cytogenetic injuries: chromosome aberrations of various types. During the acute phase of radiation sickness, the dying cells are replaced by viable ones by intensive reproduction of the small proportion of cells which have remained undamaged. The fate of the organism thus depends on whether the undamaged cells are capable of compensating for the massive destruction of the damaged ones in time. Clearly, one direct way of increasing the survival rate, as well as mitigating the severity of the radiation sickness, is to increase the number of undamaged

cells. This can be accomplished either by introducing into the organism a supplementary quantity of unirradiated cells or by raising the survival rate of the damaged cells.

Although the first method--inoculating the irradiated organism with a suspension of unirradiated cells of the hemopoietic system--gives results which are encouraging if we disregard all the difficulties of avoiding tissue incompatibility, it is hardly the most rational. In fact, due to the existence in the organism of regulatory systems which inhibit the reproduction of 'superfluous' cells, the substitution of inoculant cells for damaged ones is possible only after the onset of massive destruction of the latter. Consequently, this approach is purely therapeutic and does not prevent the development of radiation sickness, although it can sharply reduce the death rate of the irradiated organisms.

From this point of view the most rational course is to reduce the harmful effects of radiation on individual cells of the organism. Here again there are two paths: (1) radiochemical protection and (2) postradiation restoration of damaged genetic structures.

The question of the reversibility of cytogenetic radiation injuries has been discussed in the literature for some time (refs. 7 and 11). In the past decade, classical concepts regarding the reconstitution of the original chromosome structure in the process of fragment recombination have been supplemented by a number of new propositions. Proofs have been advanced that during exposure to radiation latent or potential injuries arise which may then be eliminated or realized in true breaks (refs. 3, 8 and 12). It is usually considered that radiation causes both reversible damage and damage which is in principle irreversible (refs. 5 and 6) and that, in addition, in the very process /453 of chromosome regeneration by adhesion of fragments, rearrangements of a lethal kind inevitably accrue. However, to what degree the total number of cytogentic injuries are reversible remains unclear.

Demonstration that cytogenetic injuries result from the direct passage of an ionizing particle through the cell nucleus led to the quite widespread opinion that irradiation of cytoplasm plays no role in genetic radiation effects. Nonetheless, it is well known that a number of external factors, which alter the functional state of the cell, can considerably modify the extent of genetic radiation damage. This can only be explained by supposing that cytoplasmic structures participate in the restoration of genetic structures. But the degree to which radiation damage to the cytoplasm affects the chromosome restoration process and therefore the total picture of radiation genetic injuries remains unclear.

To solve these problems it is necessary first of all to conduct a quantitative analysis of certain general patterns in postradiation cell regeneration. Yeast cells can be studied in direct regeneration experiments; as Korogodin has shown (ref. 2), these cells, when placed in water immediately after irradiation, are regenerated to a considerable extent. Although chromosome aberrations cannot be recorded immediately in yeast, it is otherwisc a convenient object of study. Radiation damage to polyploid yeast cells occurs

as a result of the growth of dominant lethals (ref. 9) and exhibits an extremely close resemblance to cell damage in mammals (ref. 10). At the same time, yeast cells are less capricious and create more opportunities for experimentation. In the stable phase they are sufficiently homogeneous for precise description by statistical laws.

Experiments were conducted on the tetraploid strain of 16 x 32 aa αα Saccharomyces cerevisiae, developed by Efrussi. A 3 day yeast culture was washed off of suslo-agar and irradiated on a GUT-Co^{60}--400 at a dose power of 1,300 rad/min. The temperature of the suspension was held at 4° to prevent regeneration during irradiation (ref. 2). Then part of the cells were placed on suslo-agar for subsequent determination of survival rate by the macrocolony method, while another part was preliminarily kept for various periods in water at 30° for study of the dynamics of the regeneration process.

When a population of irradiated cells is held in water, the number of damaged cells is in the course of time reduced, because the process of regeneration sets in. Let t be the time the cells are held in water; we shall take the initial number of damaged cells at t = 0 as unity. Then, in the general case, the fraction of damaged cells will drop by the law /454

$$\omega(D, t) = e^{-\int_0^t v(t)dt}, \qquad (1)$$

where ω(D,t) is the fraction of damaged cells irradiated with a dose D (in rad) and held in water for t hour; and v is the regeneration rate of the damaged-cell population, or the probability of regeneration of a single cell in a unit of time.

Experimental data showed that v does not depend on t from 0-24 hours, i.e.,

$$\omega = e^{-vt}. \qquad (2)$$

After 24 hours, v drops, but along with this the unirradiated cells in the control begin to die. Thus, the duration of the restoration period was limited in our tests by the length of time it is physiologically possible for yeast cells to remain in water, and in this work we restrict ourselves to examining restoration during the first 24 hours. We should remember that analogous data have been obtained for other strains.

If equation (2) were correct for an indefinitely large interval of time, the fraction of damaged cells would approach zero. This indicates that if the regeneration rate is constant, all cells without exception enjoy the possibility of regeneration and retain this possibility unaltered up to the moment of transition of cytogenetic damage to irreversible form. This transition, or realization of damage, occurs in all probability in the last stages of the cell

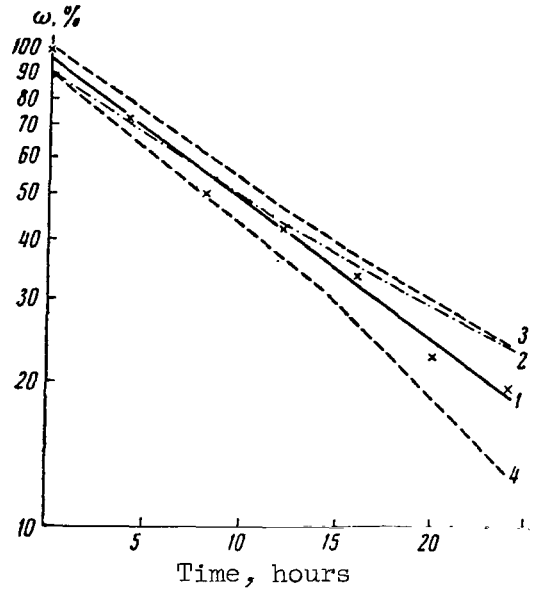

Figure 1. Possible fraction of irreversible component in regeneration of yeast cells. Abscissa, time cells were held in water; ordinate, fraction of damaged cells (from initial quantity); 1, line of regression, extended through experimental points by method of least squares; 2, possible line of regression, admitting existence of irreversible component; 3 and 4, limits of confidence interval at $p \leq 0.05$.

cycle, setting in after the cells have been seeded on the nutrient medium. The absolute reversibility of cytogenetic injuries does not square with classical concepts of regeneration, either that of the reintegration of fragments by a process of free recombination, or that of the origin of injuries irreversible in principle. This circumstance requires experimental estimation of the probable fraction of the irreversible component, since practically speaking all the cells in the tests cannot be regenerated, inasmuch as there is a limit to the time the cells can remain in water without substantial change in their physiological state.

Figure 1 presents data showing that 19 percent of the cells remained unrestored by the 24th hour. Had the line connecting experimental points been absolutely straight, this would have denoted the total absence of a component irreversible in principle. However, a small curvature is admissible in the line within the error limits for log $\omega(D, t)$. In estimating the probable fraction of the irreversible component, we must begin with the fact that its magnitude ω_∞ is not time-dependent, whereas the reversible component declines

exponentially with time. At the initial moment, when regeneration has /455
not yet begun, the sum of the reversible and irreversible components--i.e., all damaged cells--is unity

with $t = 0$ $$\omega_\infty + \omega_{rev} = 1, \qquad (3)$$

where ω_{rev} and ω_{∞} are, respectively, values of the reversible and irreversible damaged cells. The longer regeneration continues, the stronger must be the effect of the irreversible component on the shape of the curve log $\omega(D, t)$. Accordingly, in our case regeneration was traced for the maximum time, up to T = 24 hours. For this time interval we may write

with t = T $$\omega_T = \omega_{\infty} + \omega_{rev} \cdot e^{-vT} \quad (4)$$

where $\omega_T < 1$ because of reduction of the reversible fraction of the damaged cells. Inasmuch as ω_{∞} does not change with time, while ω_{rev} is the initial fraction of reversibly damaged cells, then, excluding ω_{rev} from /456 equation (3) for t = 0 and from equation (4) for t = T, we obtain

$$\omega_{\infty} = \frac{\omega_t - e^{-vt}}{1 - e^{-vt}}. \quad (5)$$

Of the possible series of values for ω_T within the limits of statistical scattering we have to postulate $\omega_T = 0.22$, which corresponds to the upper boundary of the confidence interval for $p \leq 0.01$ and T = 24 hours. We can see that the component which is irreversible in principle does not exceed 5 percent. There are no grounds for the assertion that the actual fraction of cells irreversible in principle really approaches this value; it is possible that it is much lower.

Thus, at the very least the overwhelming majority of genetic injuries of tetraploid yeast cells is in principle reversible. Consequently, this kind of cytogenetic injury can be regarded only as latent potential breaks, the elimination of which leads exclusively to restoration of the initial chromosome structure.

A second conclusion which emerges inescapably from the constancy of the regeneration rate is the "whole cell" nature of the elimination of potential injuries. In fact, the regeneration rate of the damaged population may be independent of time if and only if all cells are units of regeneration with an equal probability of restoration, independently of the number of injuries arising in them. It is clear that the cells irradiated by a single dose will always have, in accordance with the principle of hits, a different number of genetic injuries distributed in accordance with Poisson's law.

However, inasmuch as there is an inevitable spread of experimental data, it is essential to evaluate how substantial the difference is between the

postulated "whole-cell" regeneration and possible local or "independent" restoration of separate genetic structures (chromosomes) within a cell.

The basic difference between local and whole-cell regeneration is that in the first instance the average number of injuries to the cell diminishes with time, and consequently the restoration rate must increase. If it is assumed that the average number of primary injuries diminishes according to the law

$$m_t = m_0 e^{-pt}, \tag{6}$$

where m_0 is the initial number of primary injuries on the average per cell,

and p is the probability of elimination of one injury per unit of time, then we can show that the fraction of damaged cells will drop according to /457 the law

$$\omega(D, t) = \frac{\left(1 - e^{-\frac{m_t}{k}}\right)^k}{\left(1 - e^{-\frac{m_0}{k}}\right)^k}, \tag{7}$$

where k is the number of genetic structures which must be injured for the cell to die (number of targets).

Inasmuch as the curves in $\omega(D, t)$ for local and whole-cell regeneration differ least of all at $k = 1$, it was precisely this most unfavorable case which was considered when comparing the experimental curve and the theoretical curve for local regeneration with a single initial rate (fig. 2). The divergence of the curves increases with time and becomes statistically reliable after 8 hours ($p \leq 0.01$). The evaluation made below is for 24 hours, the period for which regeneration without substantial changes in the physiological state of the cells can be studied.

Within the limits of statistical scatter of the data, we can assume that in the experiment the restoration was not purely whole-cell in nature, but also partially local. Then the fraction of cells with whole-cell /458 structure regeneration can be determined as

$$a(t) = \frac{\omega_3(t) - \omega_5(t)}{\omega_1(t) - \omega_5(t)}, \tag{8}$$

where ω_1 is the fraction of cells remaining damaged during purely whole-cell restoration, ω_5 is the fraction of cells remaining damaged during purely local

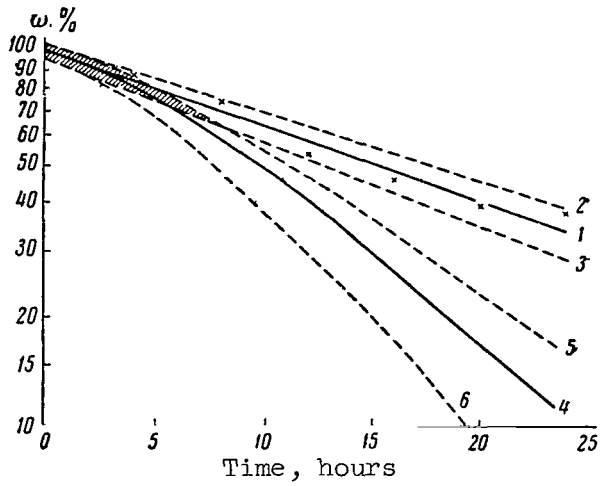

Figure 2. Comparison of experimental curve of dynamics of regeneration of yeast cells (1) with theoretical curve for local regeneration (4). Abscissa and ordinate, same as in figure 1. 1, Line of regression, extended through experimental points by method of least squares; 2 and 3, limits of confidence interval at $p \leq 0.05$; 4-6, theoretical lines for local regeneration corresponding to initial values for lines 1-3, respectively.

regeneration, and ω_3 is the fraction of cells remaining damaged during mixed restoration within the limits of the confidence interval for the experimental data ($p \leq 0.01$). For T = 24 hours, the fraction of whole-cell regeneration will equal

$$a(T) = \frac{0.30 - 0.16}{0.34 - 0.16} \simeq 0{,}87.$$

With experimental error taken into account

$$a = (87 \pm 10)\%.$$

Thus, in the majority of cells the injured structures are regenerated for the whole cell, i.e., all genetic damage is eliminated simultaneously, independently of the number of injuries. The fraction of local restoration remains within the limits of experimental error, although it cannot be completely ignored.

The continuity in time of the regeneration rate of yeast cells in water leads us to suggest the dependence of this parameter on the radiation dose

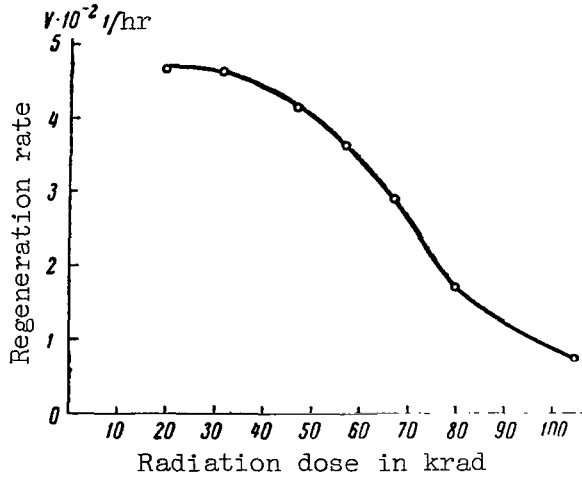

Figure 3. Dependence of regeneration rate on radiation dose.

(fig. 3). As can be seen from the graph, the restoration rate drops /459 with an increase in dose. This decline cannot be explained by an increase in the fraction of the irreversible component, since a rectilinear dependence of

$\log \omega = f^{(t)}$ is always observed over a broad range of doses. Nor can it be explained by a reduction in the probability of cell regeneration due to increase in the number of genetic injuries arising in the cells, since it has been shown above that the regeneration probability is independent of the number of injuries to the genetic structures in cells irradiated with a single dose.

These considerations, however, are valid only with a small average number of injuries whose distribution among the cells follows the law for the distribution of rare events. Now let us examine not only genetic but all injuries to the cell, most of which is filled by cytoplasm with massive, repetitious structures. If the average number of injuries is large, cell injuries will be much more homogeneously distributed among them. We may state that for a single dose in the dose range used, in the present work, the cytoplasm of different cells is injured in practically the same manner. At the same time, the degree of such massive damage will depend strictly on the radiation dose.

In this way, the dependence of regeneration on dose may be satisfactorily explained--however, only if it is assumed that the probability of the regeneration of genetic structures declines with radiation injury to massive components of the cell. Then the conclusion follows from the data cited that the restoration of genetic structures is accomplished by the entire cell functioning as a system, and the degree of radiation damage to the cytoplasm can be judged from the change in the rate of regeneration of the genetic structures.

The experiments have shown that radiation-genetic injuries to yeast cells of the dominant lethal type are largely reversible and are eliminated by whole

cells with the participation of massive cytoplasm structures. It should be noted here that the whole cell nature of regeneration was demonstrated by N. V. Luchnik in chromosome fragmentations in plant cells. It has also been shown that radiation acts independently on the formation of chromosome breaks and on the capacity of the fragments to reunite (ref. 13). These circumstances provide a basis for hoping that the observed phenomena have a fairly universal character. Further studies of this kind in our opinion must be directed toward investigating those stages of the cell cycle during which the regeneration process occurs with greatest speed, and then toward uncovering those biochemical reactions in the cell which secure restoration.

However, even at this stage it is possible to state that a significant fraction of cytogenetically damaged cells can, in principle, be re- /460
generated. It is entirely possible that painstaking study of cell regeneration will be rewarded with success in developing measures for the prompt and rational prophylaxistherapy of radiation sickness, a success which at the same time will represent an essential step along the path toward biological protection against ionizing radiation.

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SIGNIFICANCE OF THE PROCESS OF POSTRADIATION REGENERATION OF GENETIC STRUCTURES FOR THE RADIOSENSITIVITY OF CELLS

COMMUNICATION 2. RADIOSENSITIVITY OF YEAST CELLS OF VARIOUS PLOIDY

V. S. Barsukov, O. V. Malinovskiy and N. M. Mityushova

In the preceding report we described the basic quantitative regularities of yeast cell regeneration in water. It is logical to assume that /461 (1) the restoration of irradiated cells occurs not only in water, but also in the nutrient medium after the cells are seeded up until they reproduce; and (2) the rates of regeneration, in water and in the nutrient medium, although they differ in absolute magnitude, possibly depend in both cases on the radiation dose and on the yeast strain used, i.e.,

$$v_{nm} = kv_w, \qquad (1)$$

where v_{nm} and v_w are the regeneration rates in the nutrient medium and in water, and k is the coefficient of proportionality, which in the first approximation can be considered independent of the radiation dose and the yeast strain.

Then, if the cells are seeded on the nutrient medium immediately after irradiation, the population of cells making up the macrocolony will consist of cells which have escaped damage or have eliminated their injuries by the process of regeneration. Would it be possible to evaluate in this manner the fraction of restored cells among those surviving at a given dose?

In principle this can be achieved by employing a collection of genetically comparable strains of various ploidy from two genomes and more, since the number of initially arising injuries of the dominant lethal type is, according to the principle of bull's-eyes, strictly proportional to ploidy. In fact, if the restoration component of the total survival is negligibly small and the surviving mass consists almost exclusively of undamaged cells, survival /462 rate will be strictly dependent on ploidy for any values of the measured regeneration rate. Conversely, if the magnitude of regeneration component is great, and surviving cells consist largely of initially damaged, but restored cells, there must exist some relationship between the survival rate and the rate of restoration.

We should note that the literature contains no unambiguous concept of the relationship between radiosensitivity and ploidy. A number of authors, on the basis of such general parameters as growth rate and survival rate of multicellular organisms, have asserted that radiosensitivity declines with increased ploidy (refs. 3 and 6). However, it is possible to doubt the genetic comparability of the objects of study cited in these works. The most irreproachable from this standpoint are studies of polyploid series of yeast cell cultures. It has been shown that radiation death of haploids is basically the result of recessive lethals, while that of diploids and polyploids is brought about by dominant lethals (refs. 8 and 9). According to Mortimer's data, the radiosensitivity of cells increases, in full agreement with the target theory, when ploidy is increased above two genomes. However, in the study of Lucke and Sarachek (ref. 7), which utilized a different series of strains, no such simple relationship was found. An investigation of the frequency of chromosome aberrations among buckwheat diploids and autotetraploids showed few aberrations among the autotetraploids, explaining this by physiological protection of chromosomes (ref. 2).

It is entirely possible that such divergence of results is related to the fact that these works recorded only the total number of injuries without inclusion of quantitative data on regeneration.

We have therefore attempted in the present work to clarify the question of whether differences in the radiosensitivity of yeasts of different ploidy are to be explained by differences in the intensity of the regeneration process; and, if this turns out to be true, to make an approximate estimate of the relative proportions of undamaged and regenerated cells in the surviving population.

The tests were made on diploid and tetraploid strains of Saccharomyces cerevisiae yeasts of two series developed independently by B. S. Efrussi and R. K. Mortimer (abbreviated as strains Ef and M). Ploidy was monitored by chemical determination of the DNA content of the cell. This is the most reliable index of ploidy for yeasts; when our data diverged from Mortimer's conclusions, which were based on indirect indices obtained from hexaploid strain, preference was given to the chemical analysis data. However, in the present work, as will become evident further on, this correction had no significant effect. A brief characterization of the strains is given in table 1.

The method used to irradiate the yeast, and to determine survival /463
rate and rate of regeneration, have been described in the first of these two reports.

Comparison of the dose-survival rate curves for diploid and tetraploid yeasts of the two series shows that in the Ef series the tetraploid cells are more stable, and in the M series less stable, which agrees fully with the data in references 8 and 9. The presence of such directly contradictory data, obtained under the same conditions for different series of strains, shows that for practical purposes there is no immediate relationship between radiosensitivity and ploidy.

TABLE 1

Strain	Strain index	Ploidy according to authors	Ploidy according to DNA
2 Ef	WY = 110 aa	2	2
4 Ef	16 x 32 αα aa	4	4
2 M	x 324	2	2
4aM	x 323	4	4
4bM	x 362	6	4

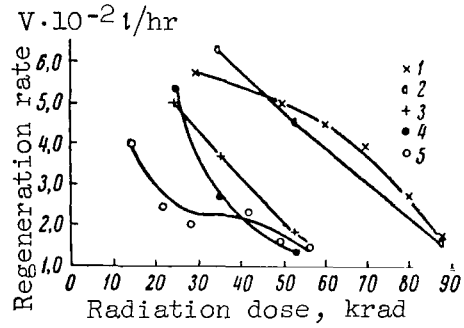

Figure 1. Curves of dependence of survival rate of yeasts of various ploidy on gamma-radiation dose. Strain designations: 1, 4 Ef; 2, 2 M; 3, 4aM; 4, 4bM; 5, 2 Ef.

Figure 2 presents data on the dependence of the restoration rate in water on the radiation dose for the same yeast strains. Both series, just as with survival rate, show contradictory relations of regeneration rate to ploidy for the whole range of doses. Among the Ef strains, the tetraploids have a considerably higher regeneration rate than the diploids; among the M strains, the reverse is true. Table 2 cites data regarding survival rate and restoration rate for various strains exposed to a single 40 krad dose. /464

There is a quite evident--and not accidental--positive correlation between the regeneration rate in water and the survival rate determined from cells seeded on a nutrient medium immediately after irradiation. It is obvious that the proposition that cell restoration occurs on the nutrient medium, just as in water, at a rate which depends on the strain and radiation dose, is true. We must mention that tests on haploids, not discussed here, failed to reveal /465 noticeable postradiation regeneration, but did show an almost total congruence of the dose-survival rate curves for strains of both series.

It is also of some interest that, given identical survival rates, yeasts of various strains differ little in their restoration rates (table 3). This

TABLE 2

Strain	Survival rate, %	Regeneration rate x 10^{-2}
2 Ef	1.5	1.3
4 bM	5.1	1.2
4 aM	11.0	2.2
4 Ef	50.0	4.8
2 M	50.0	5.0

TABLE 3

Strain	Dose, krad	Survival rate, percent	Regeneration rate x 10^{-2}
2 Ef	12.5	50	3.5
4 Ef	40	50	4.8
2 M	40	50	5.0
4aM	18	50	5.0
6bM	14	50	6.2

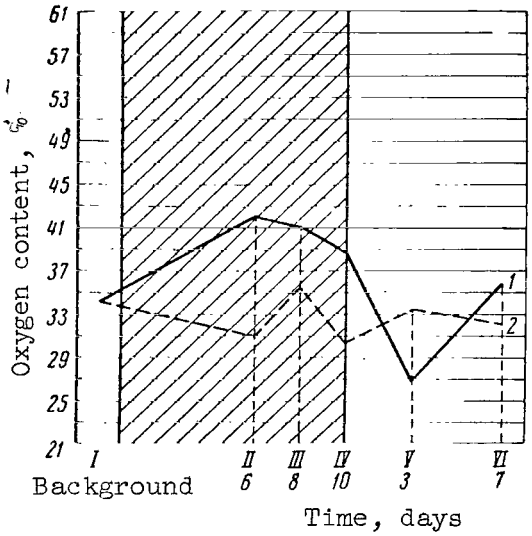

Figure 2. Curves of dependence of regeneration rates of yeasts of various ploidy on gamma-ray dose. Abscissa, radiation dose in krad; ordinate, regeneration rate x 10^{-2}. Strain designations same as in figure 1.

emphasizes the relation between survival rate and regeneration rate independently of cell ploidy. There are no grounds for assuming any definite dependence of the disposition of cells toward regeneration of genetic structures on ploidy, since the process of restoration is supported by the cytoplasm and its rate depends on the extent of radiation damage to the cytoplasm. In this case, ploidy may exert only an indirect effect upon regeneration, just like any other genetic factor.

Now let us turn to evaluation of the proportion of regenerated cells in the total of those surviving irradiation. Evidently this can be determined as

$$E(D) = \frac{S(D) - S_0(D)}{S(D)}, \qquad (2)$$

where S(D) is the survival rate measured in the experiment and $S_0(D)$ is the survival rate corresponding to the quantity of primary injuries, i.e., unmodified by restoration.

We do not know the absolute value of $S_0(D)$, but we can employ the relative value for cells of various ploidy, since in conformance with the principle of bull's-eyes, the number of primary injuries depends unambiguously on ploidy. Thus, given any fixed dose, the survival rate of tetraploids corresponding to primary unregenerated injuries is approximately equal to the square of the survival rate for diploids, that is to say, the number of survivals resulting from undamaged cells was lower for the tetraploids than for the diploids. At the same time, the overall survival rate of series Ef tetraploids in the experiment was higher than that of the diploids. According to our hypothesis, this is because of the considerably higher regeneration rate of series Ef /466
tetraploid cells. Then what must be the proportion of restored tetraploid cells among the total surviving any given dose, if such a relation between tetraploid and diploid survival rates is to occur? If it is assumed that diploid cells do not regenerate at all and that their survival rate is due solely to undamaged cells, then the estimate will be minimal, with equation (2) rewritten as

$$E_{4Ef}(D) = \frac{S_{4Ef}(D) - S^2_{2Ef}(D)}{S_{4Ef}(D)}. \qquad (3)$$

Basing our estimate on the survival rates for a dose of 12.5 krad, which corresponds to a diploid survival rate of 50 percent and a total tetraploid survival rate of 90 percent, we obtain

$$E_{4Ef}(12.5) = \frac{0.9 - 5.2^2}{0.9} = 0.72.$$

In this manner the minimum estimate of the proportion of regenerated cells among the survivals for series Ef tetraploids is 72 percent, out of a total survival rate of 90 percent.

It is interesting to determine the magnitude of the regeneration rate for a nutrient medium capable of giving the value cited for $E_{4Ef}(D)$. In accordance with the hypothesis put forth at the beginning of this report, the quantitative laws of regeneration in a nutrient medium and in water are the same. Then the proportion of damaged cells in relation to their initial number is written

$$\omega(D, T) = e^{-v_{nm}T} = e^{-kvT} = \frac{1 - S(D)}{1 - S_0(D)}, \tag{4}$$

where v_{nm} is the rate of regeneration on the nutrient medium, T is the time required for regeneration in the nutrient medium and k is the coefficient of proportionality between regeneration rates in water and in the nutrient medium.

From (4) we obtain

$$E(D) = \frac{S(D) - S_0(D)}{S(D)} = \frac{[1 - S_0(D)] \cdot (1 - e^{-vkT})}{S(D)} \simeq \frac{Vkr \cdot [1 - S_0(D)]}{S(D)}. \tag{5}$$

From this equation we can determine k, postulating Tx = 2 hr and v = 0.047 hr (in conformity with the data in figure 2). S(D) and $S_0(D)$ have already been used in estimating E(D), which was $\simeq$ 0.72 at a dose of 12.5 krad.

$$K = \frac{E(D) \cdot S(D)}{vr[1 - S_0(D)]} = \frac{0.72 \cdot 0{,}9}{0.047 \cdot 2 \cdot (1 - 0.5^2)} = 9.2.$$

Evaluation therefore shows that the regeneration rate must be approximately an order of magnitude higher in the nutrient medium than in water. /467

Analysis of equation (5) shows that the proportion of regenerated cells among total E(D) survivals varies with the dose. At D = 0, this proportion is zero. As the dose increases [1 - S_0 (D)], the proportion 1, but cannot exceed it, so that the value of E(D) is determined by the relationship v(D)/S(D), since k and T may be considered independent of dose in the first approximation.

From the experimental data it is evident that as the dose increases, the survival rate drops much faster than the regeneration rate; therefore, $v(D)/S(D)$ increases rapidly (i.e., the specific proportion of regenerated cells to the total number of survivals increases).

If we take k as 9 and the restoration period as 2 hr for all strains, then at doses corresponding to a survival rate of not less than 50 percent practically all survivals are attributable to regeneration and not to cells which have escaped primary injury.

Further, if we suppose the very worst regeneration conditions (k = 1, the generation rate in water and the nutrient medium being equal) and replace $S(D)$ by $S_0(D)$, which unquestionably further lowers the estimate, then taking $S(D)$ as, say, 10 percent, we can see that $E(D)$ for the 4Ef strain under these conditions can be written

$$E(D) \simeq \frac{0.05 \cdot 2(1-0.1)}{0.1} \simeq 0.9.$$

This predictably strong reduction in the estimate shows that for large doses giving a survival rate of less than 10 percent, in every case the overwhelming majority of surviving cells are regenerated cells.

In the preceding report it was shown that the probability of regeneration does not depend on the number of injuries to the genetic apparatus of the cell. In the present report we have shown that survival rate is practically independent of ploidy and the number of primary cell injuries corresponding with ploidy. Consequently, for doses giving a sufficiently high proportion of cells with primary damage, the fate of the population is determined by the intensity of the regeneration process and depends chiefly on the degree of radiation damage to the cytoplasm which promotes restoration.

According to a number of data, the LD_{50} of mammalian cells, judged by their capacity for normal reproduction, lies in the 50-100 rad range (refs. 4 and 10). Nonetheless, a whole organism can survive at doses which exceed this value by almost an order of magnitude. Consequently, the viability of /468 the organism is determined by an infinitesimal surviving fraction of the cells of radiosensitive organs, not exceeding tenths of a percent. It is well known that mammalian cells are capable of recovering from cytogenetic injuries (ref. 6). Starting from this fact and our experimental data, it is natural to suppose that the fate of an irradiated organism is determined mainly by regenerated cells and not by cells which have avoided radiation damage. It follows therefore that searches for agents to enhance the radiation resistance of organisms must act by increasing the rate of regeneration or prolonging the period during which rapidly dividing cells of radiosensitive organs are capable of being regenerated.

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ELIMINATION OF INJURIOUS EFFECTS OF BETA RADIATION ON SEEDS OF CULTIVATED PLANTS BY MEANS OF PHYSIOLOGICALLY ACTIVE COMPOUNDS

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Higher plants are an inseparable link in a closed ecological system. /469

When this system functions on board a spacecraft or planetary and interplanetary stations, plants will be exposed to some external factors whose effects on the ground, which is shielded by the atmosphere, are weak or nonexistent.

One of these factors is large doses of ionizing radiation. Since plants will be shielded from the external environment, the primary cosmic radiation will be transformed. Other kinds of inhibitory radiation may therefore appear. Thus, plants will be exposed to mixed ionizing radiations. Among the components of the total ionizing radiation will be gamma and beta rays.

A great deal of factual material has now been gathered on the effect of ionizing radiation on higher plants.

All kinds of ionizing radiation inhibit plants (ref. 1), even in small doses (ref. 2).

Some investigators have noted that small doses may also have a beneficial effect (refs. 3-5).

It has been experimentally shown that the various kinds of ionizing radiation affect the growth and development of plants in different fashion. A dose of 2,000 r of X-rays was found to be just as inhibitory as P^{32} irradiation with an intensity ranging from 0.1 to 10 μc/mg (ref. 6).

Some data are also available on the protective action of such factors /470 as humidity, oxygen content, low temperatures and protective compounds, e.g., polyvinylpyrrolidone (ref. 7), cystine (ref. 8), gaseous nitrogen (ref. 9), etc.

This article describes the results of experiments on elimination of the injurious effects of large doses of beta radiation on the seeds of cultivated plants by means of chemical compounds and native fluids of antibiotics.

The field method was used with the radioactive isotope of phosphorus P^{32} as the source of beta radiation.

Studies were made on corn, lupine, turnips and winter wheat. The following were tested as protective agents:

N_1-(pyridyl-(2)) N_4-(2"-oxy-1", 4"-naphthoquinone-(4)) sulfanilamide (compound P-46);

4,4-di-trichloroacetyl-diaminodiphenyl sulfone (compound G-7); 2-ketononanoic acid (compound 2149); the cultural fluid of the antibiotics 070 and 1669.

The seeds of these crops were treated in the following fashion. Half of them were soaked for 2 hours in a solution of radioactive phosphorus with an activity of 2 μc/liter. The seed: solution ratio was 1:1 (by weight). The seeds were then dried slightly and transferred to 0.01 percent solutions of the compounds in which they were soaked for 2 more hours.

The remaining seeds were immediately placed in solutions of the compounds where they were kept for 4 days. The seed: solution ratio was also 1:1.

The control was seeds soaked in water. The experiments were performed on moderately cultivated soil in a 4- to 5-fold replication. The record plot was 20 m^2 in size.

The results of the experiments on the individual crops are presented below.

Corn (Krasnodar Hybrid 1/49)

Treating seeds with radioactive phosphorus reduced the weight of the foliage (table 1).

Additional treatment not only eliminated the harmful effect of ionizing radiation, but also increased the yield above that of the variant in which the seeds were soaked in water.

Treating the seeds with solutions of radioactive phosphorus and compounds G-7, 2149 and 070 resulted in higher yields than when the seeds were treated only with solutions of the compounds.

TABLE 1. EFFECT OF SOAKING CORN SEEDS IN SOLUTIONS OF CHEMICAL COMPOUNDS AND RADIOACTIVE PHOSPHORUS ON WEIGHT OF FOLIAGE.

Variant	Wt of foliage from plot (20 m^2)			
	Without P^{32}		With P^{32}	
	in kg	in %	in kg	in %
H_2O	27.3	100.0	24.8	100.0
P-46	49.5	181.3	33.7	135.9
070	33.4	122.4	34.8	140.4
G-7	26.8	98.2	34.1	137.5
2149	40.0	146.6	38.5	155.3

Lupine /471

The results of the experiment show that lupine was less responsive than corn to ionizing radiation and the chemical compounds (table 2).

TABLE 2. EFFECT OF SOAKING LUPINE SEEDS IN SOLUTIONS OF CHEMICAL COMPOUNDS AND RADIOACTIVE PHOSPHORUS ON WEIGHT OF FOLIAGE AND GRAIN. (data cited for 20 m^2)

Variant	No. of plants		Wt of foliage				Wt of grain			
	Without P^{32}	With P^{32}	Without P^{32}		With P^{32}		Without P^{32}		With P^{32}	
			in kg	%	in kg	%	in kg	%	in kg	%
H_2O	100	121	13.0	100.0	16.9	100	1.35	100	1.24	100
P-46	83	125	11.1	85.0	17.7	105	1.09	81	1.50	121
G-7	71	135	10.2	78.5	15.8	94	0.86	64	1.40	113
2149	85	126	12.4	95.0	17.2	102	0.97	72	1.42	115
1669	82	174	14.0	108.0	16.0	95	1.14	85	1.60	129

Turnips

Treating turnip seeds with solutions of P^{32} and chemical compounds had virtually no effect on the yield of roots and tops.

Spring Wheat

The results of the experiment are presented in table 3; P^{32} had a distinctly adverse effect as contrasted with the favorable effect produced by the chemical compounds. The weight of a sheaf in the P^{32} + 1669 variant was 79 percent /472 greater than that of the control (P^{32}). The weight of the grain was 35 percent higher.

P-46 had the most potent effect on the weight of the grain and it markedly reduced the harmful effect of the radioactive phosphorus.

However, it was impossible in these experiments to eliminate entirely the harmful effect of ionizing radiation by means of the compounds under study.

To study the residual effect of treating seeds with radioactive phosphorus and P-46, the seeds obtained from these variants were planted in soil the following year without additional treatment.

The results are shown in table 4.

TABLE 3. EFFECT OF SOAKING WHEAT SEEDS IN SOLUTIONS OF CHEMICAL COMPOUNDS AND RADIOACTIVE PHOSPHORUS ON MAKEUP OF YIELD. (data cited for 20 m^2)

Variant	No. of ears	Wt of sheaf		Wt of straw		Wt of grain	
		in kg	in %	in kg	in %	in kg	in %
Seeds soaked only in solutions of compounds							
H_2O	4,061	9.26	100	4.60	100	2.62	100
P-46	4,179	11.4	124	6.11	133	3.04	117
G-7	3,610	7.7	83	3.87	84	2.22	85
2149	4,345	7.9	85	3.22	70	2.68	102
1669	3,772	9.2	99	5.02	110	2.55	97
Seeds soaked in solutions of radioactive phosphorus and compounds							
H_2O	940	2.70	100	1.32	100	0.81	100
P-46	1,140	3.87	143	2.06	156	1.14	141
G-7	1,200	4.23	157	2.60	197	0.99	122
2149	670	2.26	84	1.30	99	0.63	78
1669	1,300	1.82	179	2.92	221	1.09	135

TABLE 4. RESIDUAL EFFECT OF TREATING SPRING WHEAT SEEDS WITH SOLUTIONS OF RADIOACTIVE PHOSPHORUS AND COMPOUND P-46. (data cited for 20 m^2)

Variant	No. of plants	Wt of sheaf		Wt of grain		Wt of grains from one ear	
		in kg	in %	in kg	in %	in kg	in %
H_2O	10,230	12.27	100.0	4.79	100.0	651.9	100.0
P^{32}	8,600	10.66	86.9	3.93	82.0	767.1	117.7
P^{32} + P-46	10,280	13.02	106.1	4.88	101.8	878.7	134.8
P-46	9,920	13.96	113.8	5.58	113.6	851.4	130.6

Thus, the results of the experiments showed that the harmful residual effect of ionizing radiation persisted even in the second generation. The weight of the grain in this variant was 18 percent below that of the control.

P-46 in the second generation completely eliminated the harmful effect of radiation. The ears were even fuller than in the variant with P^{32}.

The favorable residual effect of P-46 persisted in the second generation as well.

Our findings indicate that it is theoretically possible to eliminate wholly or in part the inhibitory effect of beta radiation by means of physiological active compounds.

They demonstrate the qualitative change in reactivity of plants after irradiation, as reflected in distortion of the reaction of irradiated seeds to certain biologically active substances.

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ULTRAVIOLET IRRADIATION OF PLANTS AS A PROBLEM IN SPACE PHYTOPHYSIOLOGY

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Among the problems of space biology those related to various factors /474
of spaceflight, especially the effect of radiation on the organism, deserve particular attention. The radiation theme was, therefore, accorded an important place at the 13th International Congress on Astronautics, held in Varna on September 23-29, 1962 (refs. 19, 21 and 22).

However, little attention has been paid to the problems of the effect of ultraviolet radiation on the organism under spaceflight conditions. As O. G. Gazenko remarks with justice in reference 2, investigation of extreme conditions (UV and other forms of radiation) affecting viability during voyages into space is one of the most fundamental tasks of space biology. Sisakyan, Gazenko and Genin have pointed out (ref. 12) that a problem of undoubted interest in connection with oxygen circulation is photochemical decomposition of carbon dioxide by strong UV radiation on copper and other catalysts. Apparently, however, a more feasible approach to oxygen circulation for prolonged spaceflights would be photosynthesis.

In connection with the problem of plant photoenergetics, which we have developed, it occurs to us that the effects of UV and IR radiation must be considered. For example, inattention to the biological effects of UV radiation is basically a matter of the absence of studies of its effect on plants. The genetic and generally injurious action of UV radiation has been known for some time; however, little is known about the positive effect it has on plants.

The aim of the present work is the investigation of the effects of /475
UV radiation on photosynthesis apparatus of plants as this applies to space physiology of plants. Such studies are needed by space biology to work out principles of artificial environments in the cabins of spacecraft (ref. 3), i.e., the biological bases of a closed ecological system.

A plant organism carried in spaceflight can be acted on by various types of radiation simultaneously. In our experiments at Zapolyar'ye (Kol'sk Branch, AN SSSR) the plants were grown in pots and in the ground where, in addition to round-the-clock polar sunlight, they were exposed to artificial UV and IR radiation sources. The control group had only the natural, 24 hour exposure. The UV sources were EUV-15 erythemic lamps and BUV-30 bactericidal lamps. For 300 and 500 W IR irradiation, ZN-6 mirror lamps were used. The bactericide,

erythemic and mirror lamps were suspended above the plants in series at distances from the plants of 60-150, 30-100 and 20-80 cm, respectively. The distance between lamps was 30 cm.

Shortwave radiation (λ 254 mμ) comprised 80 percent of the bactericide lamp flux. Irradiations were made between 10 AM and 2 PM for periods of 10, 20, or 30 min during the first days and 2-3 hours later on. The 2 hour UV shortwave irradiation came from a distance of 60-80 cm from the top of the plant. In the majority of experiments UV and IR irradiations were made simultaneously.

Only a portion of the data obtained are presented in this article. Radiation intensity under the erythemic lamp (λ 300-320 mμ), measured by a UV meter of the All-Union Scientific Research Institute of Lighting Engineering at leaf level (various brightnesses), was 20-30 $\mu V/cm^2$; under the bactericide lamp (λ 254 mμ) it was about 10 $\mu V/cm^2$.

In conducting plant irradiation by short and medium UV waves it was as though we raised the plants into the UV conditions prevailing at various circumterrestrial heights. Inasmuch as the atmosphere, in the exemplary phrase of M. G. Kroshkin (ref. 9), is "an exceptionally dense light filter" admitting electromagnetic radiation only in two comparatively broad "windows"--30-75 cm and 1.25 cm-30 m--imitation or near-imitation on Earth of separate factors of the radiation regime of circumterrestrial space (up to the radiation belts) is an important condition in treating the problems of space biology under terrestrial conditions.

However, the alpine areas of Earth, where the radiation flux penetrating the thinnest part of the atmosphere is broader in the UV region, form /476 natural "outposts" for the study of UV effects on the organism. In our investigations, carried out jointly with botanists of the Botanical Institute of the Armenian Academy of Sciences, such an outpost was the Mt. Aragats, cosmic ray alpine station of the Physics Institute of the Armenian Academy.

Employing the photoenergetic approach, we devoted attention to chloroplasts and their pigments. Investigation of electron microscope preparations of the chloroplasts taken from plants exposed to UV radiation showed that shortwave UV alters their submicroscopic structures: dimensions, density, grain size and osmiophilic granules. Depending on the capacity for photoreactivation, there occurs a greater (beets, kohlrabi) or lesser (radish, lettuce) regeneration of injuries caused by UV radiation. Partly on this account the chloroplasts are characterized (during solar illumination) by fairly high resistance to UV radiation (ref. 15).

A photochemical effect of shortwave UV rays in the cell is caused by the high energy of their quanta and is related to the absorption of these rays by proteins and nucleic acids. Therefore, it has been believed that prolonged 254 mμ UV radiation is harmful to living matter. Our data showed that in the course of prolonged exposure, determined generally by tenths of an hour during around-the-clock (polar) illumination, 254 mμ waves do not destroy chloroplast

masses, although several plants exhibited a large quantity of broken-down chloroplasts in severely damaged tissues.

One of the proofs of the relatively high stability of chloroplasts under artificial UV radiation during the polar day was the normal and even augmented pigment content. Another proof is the phenomenon of photoreactivation, a process for which the abundance of shortwave radiation on mountains and at Zapolyar'ye is favorable.

Does the structure of chloroplasts change if, for example, the radiation conditions high in the mountains change? Radish leaves growing in the ground at 3,220 m on Mt. Aragats contained oval chloroplasts, and their grains considerably exceeded the dimensions of the osmiophilic granules. When radish was grown at the same height under glass in a hothouse, the chloroplast structure was different. Circular, areolar structures were formed in the leaves, having small grains and very much enlarged osmiophilic granules, attaining the dimensions of grains. Under the same conditions, but grown under a poly- /477 ethylene sheet, highly transparent to UV rays compared with glass, the chloroplasts were circular and dense, with very large, compact osmiophilic granules occupying the entire central part of the plastid. Photosynthesis in plants with such chloroplast structures was normal.

UV radiation, supplementary to that naturally received at 3,200 m above sea level and administered by a PRK-2 lamp (λ 290-360 mμ) daily for 10 min at an intensity of 1,700 erg/cm^2, produced an alteration in the microscopic structure of chloroplasts of radish leaves. They were oval, and the lamellae were arranged concentrically in rings, interspersed by circular osmiophilic granules. The protein stroma occupied a considerable area of the central part of the chloroplast. In a majority of cases these supplementarily irradiated chloroplasts contained a stroma which, on breaking up, was transformed into separate little lumps.

UV radiation also affects the pigment content in the chloroplasts. We observed, apparently for the first time, cases of positive multihour action not only of "B" but also of "C" UV rays on the pigment complex.

The pigments were separated chromatographically on paper with subsequent determination on an SF-4 spectrometer. Changes in pigment content, as tables 1-4 show, depend on UV wavelength, duration of irradiation, phase of plant development, etc.

In leaves of cabbage, beets, turnips, beans and peas (tables 1 and 2) the content of pigments either rises--after brief exposure--does not change or drops slightly--as was the case after more prolonged irradiation. In a number of cases, under the effects of shortwave UV radiation during round-the-clock solar illumination, a higher chlorophyll and carotenoid content was observed. However, in leaves of smother crop barley (table 2), severely damaged by UV radiation, the pigment content, especially chlorophyll, is cut almost in half. Prolonged medium-wave irradiation (λ 300-320 mμ) of plants of the same type of barley in the first phases had a positive effect on pigment accumulation

TABLE 1. PIGMENT CONTENT IN PLANT LEAVES AS FUNCTION OF RADIATION CONDITIONS AT ZAPOLYAR'YE (UV IRRADIATION BY BACTERICIDE LAMPS).

Plant and date	Test variant	Chlorophyll a		Chlorophyll b		Total		a/b	Carotin		Lutein		Violaxanthin		Carotenoid total	
		mg/dm^2	mg/g dry wt	mg/dm^2	mg/g dry wt	mg/dm^2	mg/g dry wt		mg/dm^2	mg/g dry wt	mg/dm^2	mg/g dry wt	mg/dm^2	mg/g dry wt	mg/dm^2	mg/g dry wt
Fodder cabbage July 12 Aug. 1	Polar day	15.1	26.9	8.9	15.0	24.0	41.9	1.7	0.23	0.43	0.11	0.11	0.03	0.06	0.37	0.60
	Same + UV (10 hr)	16.5	34.7	9.4	19.8	25.9	54.5	1.7	0.24	0.49	0.10	0.23	0.03	0.07	0.37	0.79
	Polar day	5.4	8.0	6.6	9.8	12.0	17.8	0.8	0.21	0.31	0.33	0.49	0.22	0.33	0.76	1.13
	Same = UV (40 hr)	5.9	7.1	7.6	9.0	13.5	16.1	0.8	0.26	0.31	0.35	0.41	0.21	0.26	0.82	0.98
Beet Aug. 3	Polar day	14.3	35.9	7.7	119.3	22.0	55.2	1.8	0.11	0.26	0.14	0.36	0.19	0.47	0.44	1.09
	Same + UV (28 hr)	11.2	16.6	10.5	15.0	21.7	31.6	1.2	0.23	0.33	0.34	0.49	0.14	0.27	0.71	1.09
	Polar day	4.6	11.3	2.3	7.1	6.9	18.4	2.0	0.13	0.33	0.23	0.56	0.16	0.39	0.52	1.28
	Same + UV (40 hr)	5.9	10.7	3.0	6.5	8.9	16.2	2.0	0.15	0.27	0.24	0.44	0.15	0.27	0.54	1.00
Aug. 13	Polar day	7.0	24.0	3.2	10.8	10.2	34.8	2.2	0.09	0.32	0.19	0.65	0.17	0.40	0.45	1.37
	Same + UV (28 hr)	7.6	25.5	4.2	14.4	11.8	39.9	1.8	0.11	0.36	0.13	0.43	0.14	0.47	0.38	1.26

TABLE 2. PIGMENT CONTENT IN PLANT LEAVES AS FUNCTION OF RADIATION CONDITIONS AT ZAPOLYAR'YE (UV IRRADIATION BY BACTERICIDE LAMPS).

Plant and date	Test variant	Chlorophyll a		Chlorophyll b		Total		a/b	Carotin		Lutein		Violaxanthin		Carotenoid total	
		mg/dm²	mg/g dry wt	mg/dm²	mg/g dry wt	mg/dm²	mg/g dry wt		mg/dm²	mg/g dry wt	mg/dm²	mg/g dry wt	mg/dm²	mg/g dry wt	mg/dm²	mg/g dry wt
Turnip July 27	Polar day	1.1	2.7	2.4	5.9	3.5	8.6	0.4	0.12	0.28	0.06	0.14	0.09	0.23	0.27	0.65
	Same + UV (12 hr)	0.9	3.2	2.7	9.5	3.6	12.7	0.3	0.13	0.44	0.07	0.24	0.15	0.53	0.35	1.21
Pea	Polar day	5.0	16.1	2.8	9.0	7.8	25.1	1.8	0.15	0.48	0.22	0.70	0.13	0.41	0.50	1.59
	Same + UV (12 hr)	4.2	14.3	2.9	10.0	7.1	24.3	1.4	0.12	0.40	0.20	0.68	0.12	0.42	0.44	1.50
Bean	Polar day	6.7	16.9	4.6	12.7	11.3	29.6	1.4	0.18	0.50	0.24	0.61	0.15	0.36	0.57	1.47
	Same + UV (12 hr)	6.6	15.0	5.5	12.5	12.1	27.5	1.2	0.18	0.40	0.31	0.69	0.16	0.36	0.65	1.45
Barley Polar-14 July 12	Polar day	13.7	35.7	6.0	15.7	19.7	51.4	2.3	0.19	0.49	0.14	0.35	0.05	0.13	0.38	0.97
	Same + UV (30 hr) damaged leaves	8.5	24.3	3.6	10.4	12.1	34.7	2.4	0.12	0.35	0.10	0.28	0.04	0.11	0.26	0.74
	Polar day + UV (30 hr) severely damaged leaves	5.8	20.7	3.2	11.5	9.0	32.2	1.8	0.09	0.33	0.09	0.31	0.03	0.12	0.21	0.72

(tables 3 and 4), growth and development (these data are not cited). A somewhat diminished effect of medium-wave UV rays on the accumulation of plastid pigments was noted in the stalk phase, where red-IR radiation was more favorable for pigment accumulation than UV. However, after IR radiation with mirror lamps there was a negative influence on the pigment content.

On the whole, medium-wave UV rays in round-the-clock polar illumination do not act negatively on barley during the stalk phase. /480

Additional IR radiation was clearly advantageous for the growth and development of barley. At 3,200 m on Mt. Aragats, supplementary medium-wave UV radiation also enhanced the productivity of barley. UV and IR radiation exert an influence on the interrelation between leaf and root nourishment of plants.

Thus, on the basis of these data regarding the effect of solar radiation on plants, it seems to us expedient to rephrase the question about the use of plants from areas with a spectrum bordering on the visible.

The normal structure of chloroplasts and much higher content of pigments even in plants exposed to a 40 hour UV irradiation (λ 254 mμ) testifies to the definite resistance of the photosynthetic apparatus to harmful UV rays. The positive effects accompanying medium-wave UV irradiation indicate that it is necessary to investigate the many facets of the UV region and its action in order to solve the problems of space biology. Studies in the area of space physiology of plants must be directed not only toward protecting plants /481 from UV radiation, but also toward using the various UV spectrum regions to increase plant resistance to other factors in cosmic radiation.

Studies at our laboratory have established that at a height of 4,000-5,000 m (Pamirs) and 3,200 m (Mt. Aragats) there is an increase in the absorption of radiation energy from UV to IR rays by plant leaves exposed to high natural UV radiation.

Measurements of spectral properties made by a photointegrating instrument in the eastern Pamirs at a height of about 5,000 m showed a greater photoabsorbing capability of plant leaves than previously observed (table 5). By virtue of their anatomicophysiological peculiarities, the leaves of plants inhabiting areas near perpetual glaciers, the upper boundary of natural growth, probably reflect and emit little radiation energy. Even IR rays are absorbed by such plants up to 1/4 or 1/3 of their radiative flux on an 860-mμ wavelength. One of the major reasons for the high absorption of IR rays by plant leaves at great altitudes in the mountains is the temperature and radia- /483 tion condition. Artificial, prolonged reduction of the UV flux of plants on Aragats induced a change in spectral properties of the leaves and diminished absorption of IR energy. On the other hand, supplementary artificial UV radiation (λ 400-860 mμ) raised leaf absorption of radiation energy by $30\text{-}40.10^3$.erg/cm^2.

TABLE 3. CHLOROPHYLL CONTENT IN POLAR-14 BARLEY LEAVES ACCORDING TO DEVELOPMENT PHASE AS FUNCTION OF RADIATION CONDITIONS IN ZAPOLYAR'YE. UV IRRADIATION BY ERYTHEMIC LAMPS, IR IRRADIATION BY ZN-6 MIRROR LAMPS.

Development phase and date	Test variant	Chlorophyll						a/b
		a		b		Total		
		mg/dm^2	mg/100 g dry wt	mg/dm^2	mg/100 g dry wt	mg/dm^2	mg/100 g dry wt	
July 3	Polar day	5.0	550	2.3	187	7.3	737	2.1
	Same + UV (10 hr)	6.0	636	2.8	294	8.9	930	2.2
	Same + IR (50 hr)	5.0	630	2.8	334	8.8	964	1.8
July 16	Polar day	11.8	762	4.6	187	16.4	949	2.6
	Same + UV (20 hr)	9.5	497	4.0	207	13.5	704	2.4
	Same + IR (60 hr)	10.7	537	4.6	229	15.3	766	2.3
Beg. of July 24	Polar day	7.4	412	4.2	234	11.6	646	1.8
	Same + UV (27 hr)	6.6	399	3.6	216	10.2	615	1.8
	Same + IR (93 hr)	6.4	382	3.6	182	10.0	564	1.8

TABLE 4. CAROTENOID CONTENT IN POLAR-14 BARLEY LEAVES ACCORDING TO DEVELOPMENT PHASE AS FUNCTION OF RADIATION CONDITIONS IN ZAPOLYAR'YE. UV IRRADIATION BY ERYTHEMIC LAMPS, IR IRRADIATION BY ZN-6 MIRROR LAMPS.

Development phase and date	Test variant	Carotin		Lutein		Viola-xanthin		Total	
		mg/dm^2	mg/100 g dry wt	mg/dm^2	mg/100 g dry wt	mg/dm^2	mg/100 g dry wt	mg/dm^2	mg/100 g dry wt
July 3	Polar day	0.13	4.6	0.12	4.2	0.07	2.3	0.32	11.1
	Same + UV (10 hr)	0.18	9.8	0.15	8.7	0.13	6.6	0.46	25.1
	Same + IR (50 hr)	0.18	10.6	0.15	9.3	0.14	8.1	0.47	28.0
July 16	Polar day	0.14	6.8	0.17	8.1	0.12	5.7	0.43	20.6
	Same + UV (20 hr)	0.13	5.5	0.14	7.5	0.12	6.8	0.39	19.8
	Same + IR (60 hr)	0.13	6.3	0.15	7.5	0.11	5.3	0.39	19.1
Beg. of July 24	Polar day	0.16	8.7	0.19	10.6	0.14	7.7	0.49	27.0
	Same + UV (27 hr)	0.13	7.9	0.17	10.3	0.10	6.0	0.39	24.2
	Same + IR (93 hr)	0.12	9.1	0.15	8.6	0.08	4.5	0.35	22.2

TABLE 5. SPECTRAL PROPERTIES OF PLANTS OF EASTERN PAMIRS 5,000 m ABOVE SEA LEVEL. MEASUREMENTS WERE MADE 11 AM - 1 PM, AUGUST 6, 1962.

Wavelength, mμ	Primula moorkroftiana			Primula turcestanica			Swertia marginata			Oxygraphis, glacialis			Saussurea sp.		
	Reflection	Admittance	Absorption	Reflection	Admittance	Absorption	Reflection	Admittance	Absorption	Reflection	Admittance	Absorption	Reflection	Admittance	Absorption
360	0.2	0.0	99.8	0.3	0.0	99.7	0.2	0.0	99.8	0.2	0.0	98.2	4.3	0.0	95.7
440	3.7	0.0	96.3	2.1	0.0	97.9	4.4	0.0	95.5	3.3	0.0	96.7	3.9	0.0	96.1
434	5.2	0.0	94.8	5.4	0.0	96.6	6.2	0.0	93.8	9.7	0.1	90.2	3.7	0.0	96.3
548	6.1	4.2	89.7	5.3	1.8	92.9	7.9	4.0	88.1	7.9	4.1	88.0	5.4	2.4	92.2
578	13.9	0.9	85.2	15.9	1.9	82.2	7.7	1.0	91.3	7.0	1.7	91.3	6.1	0.4	93.5
621	12.5	2.4	85.1	6.9	0.7	92.4	12.0	2.6	85.4	11.5	2.0	86.5	8.1	0.4	91.5
645	8.7	0.5	90.8	7.2	0.4	92.4	8.4	0.8	90.8	7.7	0.7	91.6	9.1	0.3	90.6
660	8.5	0.3	91.2	5.5	0.3	94.2	8.9	0.4	90.7	6.3	0.5	93.2	7.2	0.2	92.6
698	7.9	2.6	89.5	7.8	4.3	87.9	7.1	3.7	89.2	7.1	4.0	88.9	11.1	2.9	86.0
730	19.5	13.0	67.5	36.0	26.0	48.0	20.0	12.5	67.5	19.4	11.0	69.6	30.2	10.2	59.6
860	40.0	30.0	30.0	39.0	32.0	29.0	40.0	34.0	26.0	46.0	37.0	17.0	33.8	22.0	44.2

This is a basis for advancing the hypothesis that for plants located in the cabin of a spacecraft the composition and intensity of the radiation flux, as well as the presence or absence in it of UV and IR rays bordering on the visible spectrum, must be undifferentiated. From the point of view of the concepts which we have developed regarding plant photoenergetics, plants under extreme conditions of existence utilize radiation energy in a broader spectral range in the course of their activity.

For optimal or maximal photosynthesis and for chloroplast stability under circumstances of special radiation conditions, artificially created in the mountains or existing in a spacecraft cabin, it is probably necessary to maintain a definite relationship in the radiation flux between UV and IR radiation and between them and rays of the visible part of the spectrum. This is also essential for the processes of photoadaptation and photoreactivation, examined in connection with problems of space physiology of plants (ref. 17).

Finally, the relationships between spectral regions, during plant irradiation in a spacecraft cabin, probably have an important bearing on resistance to and reactivation under ionizing radiation (X-rays and other rays). Taking into account the phenomenon of photoadaptation and photoreactivation in relation to chloroplast resistance to UV radiation, as well as the increase with height of leaf absorption of radiation energy affecting photosynthesis and other processes, it is necessary to investigate growth organisms (for the purposes of space physiology of plants) in a broader spectral range. Such an approach must be examined beforehand by establishing a bioenergetic basis for designing and constructing a closed ecological system.

What will be the photosynthesis of plants if they spend a long time under radiation at heights of 4,000 and 5,000 m?

As our investigations, conducted with the device of Chatskiy and Slavik, show, plants growing wild in the eastern Pamirs, in the region of Chechekty, have photosynthesis within the usual "normal" limits, whereas among certain other types it is higher. This is all the more remarkable because cosmic radiation at these heights is greater than at sea level.

Taking into account the photoabsorption capability of alpine plants (table 5), it is not surprising that certain plants at heights of 4,000-4,700 m exhibit a noonday photosynthesis of 40-60 mg $CO_2/dm^2/hr$ at a radiation of 1.5 $cal/cm^2/min$. Photosynthesis among various generations of barleys aver- /484 aged 10 mg $CO_2/dm^2/hr$ (table 7).

Other wild varieties had photosynthesis, determined by C^{14} analysis, of 40-90 mg $CO_2/dm^2/hr$ (ref. 7).

Data in the literature (ref. 20) are of some interest in evaluating the quantum yield of photosynthesis in UV rays. The quantitative effect of photosynthesis was measured at 270 to 435 mμ by the polarographic method and

TABLE 6. PLANT PHOTOSYNTHESIS AT CHECHEKTY IN EASTERN PAMIRS, 3,860 m ABOVE SEA LEVEL.

Date	Plant type	Time	Air temperature, °C	Photosynthesis, $mg/dm^2/hr$
July 30	Goosefoot (Chenopodium)	17 hr 51 min	21.8	5.9
		18 hr 04 min	20.0	10.0
July 31	Goosefoot (Chenopodium)	9 hr 21 min	15.0	11.5
		9 hr 28 min	15.0	15.4
July 31	Alpine oxytropis	10 hr 05 min	15.0	10.8
		10 hr 11 min	15.0	15.6
Aug. 9	Swertia	10 hr 19 min	18.0	2.1
Aug. 9	Saussurea	13 hr 00 min	14.0	4.7
		15 hr 17 min	16.7	4.9
		17 hr 25 min	16.0	36.5
		17 hr 36 min	16.0	20.0
Aug. 9	Primrose (Primula moorkroftiana)	10 hr 46 min		5.4
		16 hr 44 min	15.0	4.8
		18 hr 05 min	12.2	1.9
Aug. 9	Grass (Lasiagrostis)	11 hr 51 min	14.0	11.6
		13 hr 12 min	13.5	10.9
		13 hr 28 min	14.0	12.0
		14 hr 57 min	18.0	0.0
		17 hr 13 min	16.0	9.2

TABLE 7. PHOTOSYNTHESIS OF VARIOUS GENERATIONS* OF NUTANS BARLEY AT PAMIRS BIOLOGICAL STATION IN CHECHEKTY, EASTERN PAMIRS, 3,860 m ABOVE SEA LEVEL. MEASUREMENTS WERE MADE ON AUGUST 3, 1962.

Generations of barley	Time	Solar radiation, $cal/cm^2/min$	Air temperature, °C	Photosynthesis, $mg/CO_2/dm^2/hr$
Original	10 hr 14 min	1.00	11.2	6.3
1st gen	10 hr 42 min	1.16	13.2	9.0
2nd gen	11 hr 14 min	1.30	14.2	9.7
3rd gen	11 hr 32 min	1.32	16.2	9.4
4th gen	12 hr 05 min	1.42	17.0	13.8
5th gen	12 hr 34 min	1.50	16.0	11.2
Original	12 hr 14 min	1.45	17.0	9.5

*The authors extend their thanks to R. Nigmatullin for the plants used in these tests.

comprised 0.067 M O_2/quantum at λ 435 mμ, 0.047 at λ 320 mμ, and 0.027 at λ 270 mμ; this method uncovered no photosynthesis lower than 270 mμ. From the point of view of utilization of radiation energy and the results of our investigations under conditions of uninterrupted illumination, our conclusion that active development of algae for correct evaluation of their productivity can be secured only during round-the-clock, sufficiently intense illumination is of importance (ref. 11). Probably, under such conditions high resistance of the photosynthetic apparatus to UV radiation can be achieved on a spacecraft without the necessity of protecting the plants against all UV radiation. /485 Furthermore, for optimum activity of the animal organism it is apparently necessary to maintain a certain level of irritant action of protein-photolysis products, arising under the influence of UV rays (ref. 1).

For evaluation of UV radiation from the point of view of space physiology of plants, the data indicating that in some plants the chloroplasts and pigments are quite resistant to UV radiation deserve attention. Shortwave UV rays at a certain level stimulate biosynthesis of pigments.

Thus, the material described in this article supports the assumption that UV radiation of plants is an important, but until now scarcely considered problem of space physiology of plants. Adequate attention must be accorded to this problem. The purpose of the present report was to draw attention to the study of the effects of UV radiation on photosynthesis and other processes of plants under conditions of cosmic UV radiation.

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VOLUNTARY CHOICE BY ANIMALS OF GAS MEDIA WITH VARIOUS OXYGEN CONTENTS UNDER NORMAL CONDITIONS AND AFTER EXPOSURE TO A HYPEROXIC ATMOSPHERE

I. S. Breslav

There are at present numerous findings regarding the effect of high /487 partial pressure of oxygen in the environment on different physiological functions of the organism. One question has received little study. It is the behavior of animals in response to a hyperoxic environment and serves as a sensitive integral indicator of changes in surrounding conditions.

We developed a method for studying the voluntary choice by experimental animals of gas media of varying composition (gas preference). With the technical assistance of A. M. Il'nitskiy, we constructed a special apparatus--a "gas ladder"--different steps of which, with the help of special devices, sustained gradual changes (gradients) in the concentration of some particular gas.[1] In these experiments, one end of the gas ladder ("air") was filled with ordinary air, while the other ("oxygen") had a hyperoxic medium containing 60 or 90 percent oxygen and 40 or 10 percent nitrogen, respectively. Naturally, the composition of the atmosphere in the middle of the apparatus contained a mean (between both ends) percentage of oxygen.

Experiments were carried out on white male mice weighing 20-27 g. Each animal was placed for 30 min into the "gas ladder," where all of its movements were recorded. Among other indicators, a record was kept of what step of the apparatus, i.e., what concentration of oxygen, was chosen by the animal after a number of orienting runs. The gas preference of each animal was deter- /488 mined in this way. Finally, the mean preference was computed for each series of experiments. The magnitude and direction of the deviation of this indicator from the mean percentage of oxygen concentration in the gas ladder made it possible to evaluate the reaction of the animals to the hyperoxic environment maintained at the oxygen end of the apparatus.

We made a study in our work of the gas preference of both intact mice and mice exposed for several days to an atmosphere with a high content of oxygen.

1. Experiments with Intact Animals

The table contains data showing how normal mice react to a hyperoxic environment. In a 60 percent O_2 concentration at the "oxygen" end of the apparatus,

[1] A more detailed description of the gas preference method is given in another report (ref. 3).

GAS PREFERENCE OF INTACT MICE UNDER CONDITIONS OF OXYGEN CONTENT GRADIENTS.

No. of animals	Oxygen concentration			Deviation of preference from mean percentage of O_2 in apparatus
	At "oxygen" end of apparatus	In center of apparatus	Preference (mean)	
45	60.0	40.0	34.5	-5.5 ± 1.1
44	90.0	55.0	42.4	-12.6 ± 3.5

we determined the statistically significant avoidance of such a moderate hyperoxic atmosphere by the animals. The animals' negative reaction to 90 percent oxygen was even more pronounced.

As is known, brief inspiration of gas mixtures containing 90 percent and 60 percent oxygen is harmless to both animals and man (refs. 10, 11, and 13). However, it is also known that, beginning with a 40-50 percent oxygen concentration of inspired air (ref. 14), a whole series of compensatory changes characteristic of hyperoxia take place in the respiratory, circulatory and other systems (refs. 4 and 9). These changes are thought to arise reflexly in response to stimulation of the carotid sinus (ref. 12) and possibly of receptor fields of the lung and the upper respiratory tract (refs. 5 and 6). It may be assumed that the negative reaction of the animals to 60 and 90 percent oxygen is induced by a similar form of reflex action.

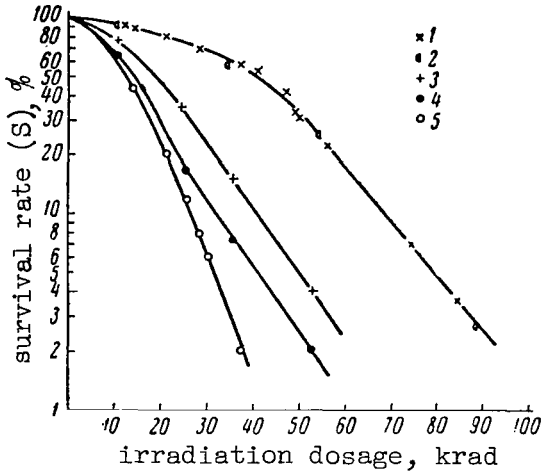
A detailed analysis of the mechanism of the behavioral reaction to a medium containing a high oxygen content might establish the correctness of this explanation.

2. Experiments with Animals Exposed for a Long Time to a Hyperoxic Medium

A special series of experiments was carried out on mice kept for 6-10 /489
days in a chamber containing a gas mixture of 60 percent oxygen and 40 percent nitrogen. A group of animals were kept in a chamber containing ordinary atmosphere as a control.

The gas preference was determined for both experimental "hyperoxic" and control mice after they had been kept for varying periods in the chambers. The investigation was conducted with 60 percent concentration of oxygen maintained at the "oxygen" end of the "gas ladder," i.e., the same environment in which the experimental mice were previously kept for an extended period of time.

It is evident that the mice kept 6 or 8 days in an atmosphere containing 60 percent oxygen chose a much higher concentration of oxygen than normal; their mean preference during this period almost coincided with the average composition of the medium (horizontal axis in the figure), thereby indicating the absence of a negative reaction to the hyperoxic environment. The animals kept under /490



Dynamics of gas preference of mice during exposure to gas medium containing 60 percent oxygen, and aftereffects: 1, before experiment; 2, 6 days after start of experiment; 3, 8 days; 4, 10 days; 5, 3 days after termination of experiment.

hyperoxic conditions for 10 days had an apparently weak negative reaction to the "oxygen" end of the apparatus. However, their preference differed significantly ($P < 0.05$) from the comparable indicator of the control group, which showed only the usual inconsequential fluctuations.

Three days after these mice had been transferred to an air environment, the reverse relations appeared. Now it was the experimental animals that particularly avoided the hyperoxic environment, choosing an even smaller concentration of oxygen than the controls. Here too, the difference between the two groups was statistically significant. Finally, on the 7th day of exposure to air the "hyperoxic" mice showed somewhat higher preference figures than the controls, but here the difference was not statistically significant.

It is possible that the changes observed in the gas preference are related to changes in the course of the oxidation-reduction processes and some other physiological functions, which take place in animals as they adapt to increased partial pressure of oxygen in the atmosphere (refs. 1, 5, and 11). These changes may, in turn, modify the oxygen requirement of the animals and thereby influence their selection of a specific concentration of oxygen in the environment.

The fact that on transfer to an air environment experimental animals select an environment more deficient in oxygen for a certain period of time may be regarded as a reflection of a reverse reorganization of the metabolic processed. Following exposure to an oxygen-enriched atmosphere, transfer of the animals to breathing air actually produced a temporary suppression of metabolism (ref. 2).

These facts, however, may also be explained by the influence of the hyperoxic environment on the central nervous system function. G. N. Zilov (ref. 5) noted a decrease in the conditioned reflex activity of dogs while breathing gas mixtures containing more than 55 percent oxygen. It is possible that in our experiments with mice subjected to a hyperoxic environment, the excitability of certain receptors and nerve centers decreased, resulting in impairment of the capacity of the animals to differentiate between different gas media.

It should be pointed out that this particular type of change in excitability was disclosed while studying defense reflexes in experimental mice. The biggest changes were noted, as in our experiments, after 6 days of exposure to an environment with 60 percent oxygen content. These changes were considerably weaker by the 10th day.

With regard to the temporary intensification of the negative reaction to the hyperoxic environment upon termination of the experiment, this may depend on an increase in the reactivity of the corresponding nerve apparatuses. /491 Such a "reverse wave" may be considered a phase phenomenon, which is partly inherent in the physiological reactions to a variety of environmental factors (refs. 7 and 8).

However, it may also be assumed that the changes in gas preference observed in the animals on their 10th day of exposure to a hyperoxic environment and during the first few days that followed were caused by the incipient pathological action of oxygen.

Further study of the gas preference of animals following more or less protracted exposure to different partial pressures of oxygen may help to elucidate the dynamics and mechanism of influence of a gas medium on the organism.

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EFFECTS OF INCREASED PARTIAL PRESSURE OF OXYGEN ON THE MORPHOLOGICAL COMPOSITION OF THE PERIPHERAL BLOOD IN ANIMALS

I. S. Breslav and A. M. Shmeleva

Breathing gaseous mixtures with increased partial pressure of oxygen /492 usually reduces the hemoglobin concentration and number of erythrocytes in animals and man (refs. 3, 5, 11 and 25). This reaction is the opposite of that observed during hypoxia, and it is aimed at restricting the transport of oxygen to the tissues. Little is known about the change in hemoglobin concentration and number of erythrocytes in peripheral blood following prolonged exposure to an environment with increased oxygen content.

Inadequate attention has been accorded to the changes in white blood cells during hyperoxia, an important indicator of general reactivity. A number of authors observed leukocytosis under these conditions (refs. 5, 9 and 16). However, persons receiving oxygen therapy have had leukopenia (ref. 14), and V. A. Alekseyev (ref. 1) failed to detect any changes in the white blood cells of persons systematically subjected to the action of oxygen under pressure.

In studying bone marrow punctates from rabbits exposed to compressed air for a long time, A. A. Bereza (ref. 3) noted an intensification of erythropoiesis combined with suppression of white marrow.

The conflicting data are probably due to the fact that the various investigators did not fully take into account the rather complex dynamics of the total content and quantitative relations of the different formed elements of the blood.

The purpose of our work was to clarify the dynamics of the indices of peripheral blood morphology in animals kept for different periods of time in an environment with increased partial pressure of oxygen. /493

Two series of experiments were performed on 360 white male mice of the 57CC strain 2-3 months old.

We first investigated the effect of relatively brief exposure to gaseous mixtures with various oxygen concentrations: (60 percent O_2 and 40 percent CO_2 for 36 hours; 90 percent O_2 and 10 percent N_2 also for 36 hours; pure oxygen at a pressure of 2.5 atm for 1.5 hours).

The second series traced the effect of prolonged exposure (up to 10 days) to a gaseous mixture consisting of 60 percent oxygen and 40 percent nitrogen.

In both series of experiments, besides the experimental "hyperoxic" group, a control group was investigated simultaneously. The latter was kept in the same chamber as the experimental group, but the oxygen content of the atmosphere was normal.

Blood was obtained for analysis by the Kleineberger and Karl method, both immediately after hyperoxia and at various intervals after it was halted. The blood of each animal was investigated only once in order to prevent complications due to blood loss. The animals were examined at various times and in successive groups.

The hematological indices are presented on the following figures in the form of mean values. The changes in blood morphology of the "hyperoxic" mice noted below were, in general, statistically significant as compared with the level of these indices in the control, except in the case of monocytes and eosinophils. Owing to the low content of these formed elements in the blood, the quantitative changes in our experiments usually remained within the limits of possible individual variations.

Immediately after 36 hours of exposure to 60 percent oxygen (fig. 1A), the experimental animals exhibited only a slight decrease in the hemoglobin concentration and elevation in the erythrocyte count. The original values were restored 3 days later, but both indices perceptibly decreased by the 7th day.

The same length of exposure to 90 percent oxygen (fig. 1B) caused a sharp decrease in the number of erythrocytes, particularly reticulocytes. The hemoglobin concentration remained unchanged at first (hence the increase in the color index), but 3 days after the experiment it too decreased. By the 7th day there was a tendency for the morphology of the red blood cells to become normal.

The effect of 1.5 hours of exposure to oxygen at a pressure of 2.5 atm (fig. 1C) was different. At first the number of erythrocytes increased; the hemoglobin concentration did so to a lesser degree, but three days later /495 the hemoglobin concentration, color index, and reticulocyte content sharply decreased. Seven days after the experiment they tended to return to normal.

Figure 2 shows the dynamics of the indices of the red blood cells in mice after 10 days' exposure to an atmosphere containing 60 percent oxygen (second series of experiments). After an initial increase, the number of erythrocytes and reticulocytes steadily decreased. By the 6th day of the experiment the hemoglobin concentration and color index decreased considerably. By the end of the experiment both hemoglobin concentration and color index increased slightly, but the number of erythrocytes and reticulocytes continued to decrease.

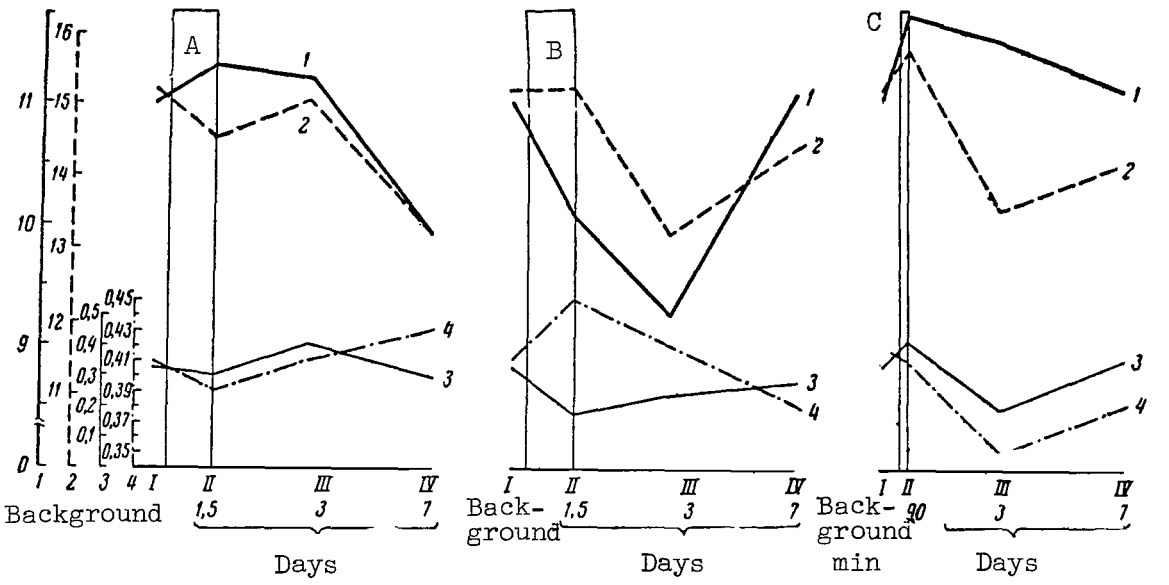

Figure 1. Changes in morphology of erythrocytes in mice exposed to gaseous mixtures with increased partial pressure of oxygen. A, 60 percent O_2 (36 hours); B, 90 percent O_2 (36 hours); C, compressed O_2 at a pressure of 2.5 atm (1.5 hours); I, before experiment; II, immediately afterward; III, 3 days later; IV, 7 days later; 1, erythrocyte content (in millions per mm^3 of blood); 2, hemoglobin concentration (in g percentages); 3, reticulocyte content (in thousands per mm^3 of blood); 4, color index.

Transferring the animals to an air medium caused the reticulocyte content to rise, although the total number of erythrocytes during the next 3 days continued to decrease. The erythrocyte count and hemoglobin concentration then increased and 7-10 days after the experiment ended, the red blood indices were more or less normal.

Regarding these changes in the red blood cells of the experimental animals, it is important to distinguish between the reactions of hemodynamic character and the changes attributable to alteration of the function of the hematopoietic organs.

It is evident from the curves in figure 1 that the most characteristic response was to 90 percent oxygen. There was an immediate suppression of erythropoiesis (decrease in total number of erythrocytes, especially in the reticulocytes, their immature forms), which was in all likelihood partly compensated in the early period by emptying of the blood depots (increase in the /496

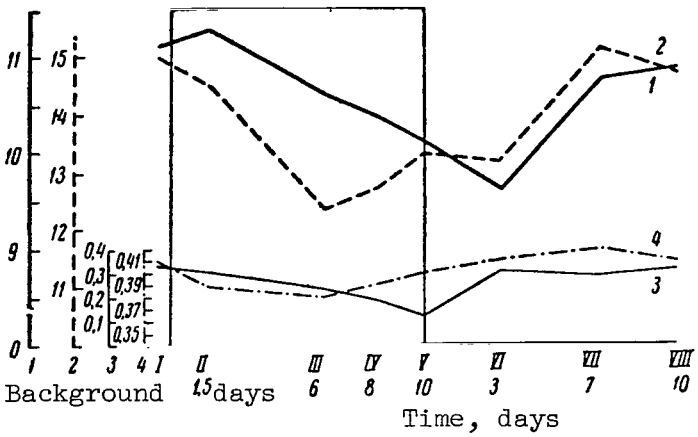

Figure 2. Changes in morphology of red blood cells in mice during exposure to gaseous mixture with 60 percent oxygen content and afterward. I, before experiment; II, 1.5 days after start of experiment; III, 6 days later; IV, 8 days later; V, 10 days later; VI, 3 days after end of experiment; VII, 7 days later; VIII, 10 days later. Designations of curves same as in figure 1.

color index). The response to 60 percent oxygen was not manifested until several days after the experiment.

The results of exposing the animals to oxygen under increased pressure were somewhat different. At first there were phenomena suggestive of redistribution of blood. Later, however, signs of temporary suppression of erythropoiesis were evident.

Many days' exposure to a moderately hypoxic atmosphere (fig. 2) at first induced a slight increase in the total number of erythrocytes with a decrease in the color index, although the reticulocyte content and hemoglobin concentration were already beginning to drop, a possible indication of the loss of water by the erythrocytes due to the movement of tissue fluid observed by some authors in persons inhaling oxygen (ref. 22). The symptoms of suppressed erythropoiesis subsequently appeared (the number of reticulocytes continued to drop as did the total content of red blood cells).

This finding is consistent with the data of other authors (refs. 6 and 20), who noted a slowing of blood regeneration after bleeding in animals subjected to increased oxygen pressure. It is also possible that the erythrocytes here had begun to disintegrate more rapidly. For example, divers systematically exposed to compressed air have developed symptoms of hemolytic jaundice along with anemia (ref. 13).

At first glance, it is hard to understand why the decrease in hemoglobin concentration turned into an increase after the 6th day of the experiment. We believe the reason is that prolonged exposure to increased partial pressure of

oxygen caused changes in the lungs with lowering of their respiratory function or with highly pronounced compensatory reactions, specifically, a rapid decrease in the hemoglobin concentration. In either case the result should have been oxygen starvation (possibly combined with hypercapnia). This, in turn, could have caused emptying of the blood depots, an indication of which was the increase in the color index.

Transfer of the animals to an atmosphere with a normal oxygen concentration signified chiefly an intensification of erythropoiesis. Air affects the organism exposed to increased partial pressure of oxygen for a long time the same way that a hypoxic atmosphere does. It is a well-known fact that /497 erythropoiesis is stimulated in oxygen starvation because of an increase in the quantity of erythropoietins in the blood (refs. 23 and 24).

Thus, a partial pressure of oxygen tends to regulate the erythrocyte content of the blood in animals. This regulatory influence is evidently realized through changes in oxygen tension executed through neuroreflex pathways (ref. 10). However, prolonged exposure to hyperoxia induces changes that can be related to the incipient pathological action of oxygen.

In the first series of experiments (fig. 3), the following changes were found in the white blood cells of experimental animals.

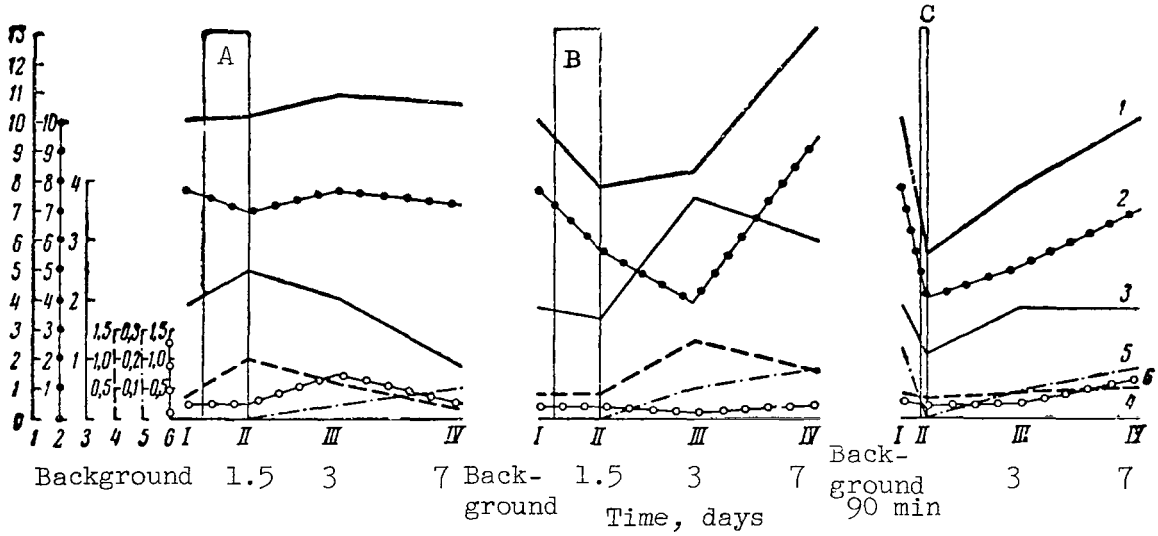

Figure 3. Changes in morphology of white blood cells in mice exposed to gaseous mixtures with increased partial pressure of oxygen. A, 60 percent (36 hours); B, 90 percent O_2 (36 hours); C, compressed O_2 at a pressure of 2.5 atm (1.5 hours); I, before experiment; II, immediately afterward; III, 3 days later; IV, 7 days later; 1, total number of leukocytes; 2, lymphocytes; 3, neutrophils (total); 4, immature forms of neutrophils (stab nuclear, juvenile); 5, eosinophils; 6, monocytes. All forms are given in thousands per mm^3 of blood.

Exposure of mice to an atmosphere with 60 percent oxygen content for 36 hours had little effect on the leukocyte count. There was only a slight decrease in the number of lymphocytes and moderate neutrophilosis (fig. 3A), which gave way to neutrophilopenia 7 days later.

Marked leukopenia due to a sharp decrease in the number of lymphocytes (which normally constitute 70-80 percent of the white blood cells in mice) followed exposure to 90 percent oxygen (fig. 3B). However, 7 days after the experiment the number of lymphocytes exceeded the original level. The period of increase in neutrophil count coincided with the peak of lymphopenia (3rd day).

Immediately after brief exposure to compressed oxygen (fig. 3C), there was a sharp decrease in the content of all forms of leukocytes, especially lymphocytes, which gradually returned to normal on the following days.

In a second series of experiments involving prolonged exposure of mice to a gaseous mixture containing 60 percent oxygen (fig. 4), there was a steady decrease in the number of lymphocytes until the 6th day. It was accompanied by a decrease in the total number of white blood cells, although the neutrophil content increased slightly during the first few days of the experiment.

The picture changed abruptly between the 8th and 10th days. The number of lymphocytes rose almost to the original level and neutrophilosis again appeared.

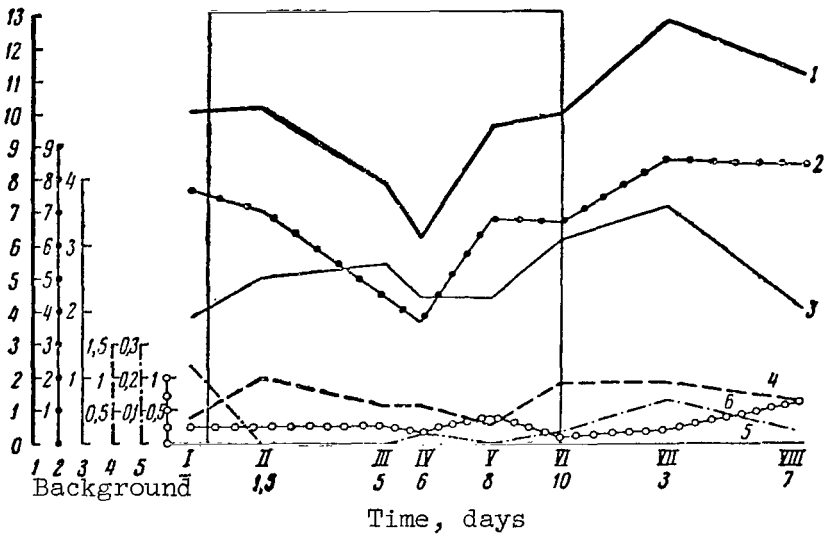

Figure 4. Changes in morphology of white blood cells in mice during exposure to gaseous mixture with 60 percent oxygen content and afterward. I, before experiment; II, 1.5 days after start of experiment; III, 4 days later; IV. 6 days later; V, 8 days later; VI, 10 days later; VII, 3 days after end of experiment; VIII, 7 days later. Designations of curves same as in figure 3.

Three days after the animals were transferred to air, moderate leukocytosis developed as a result of an increase in the number of lymphocytes and especially neutrophils. On the 10th day the content of the individual forms of the leukocytes was virtually the same as at the beginning of the experiment.

Changes in the monocyte content in our experiments were vague. They mostly tended to resemble the dynamics of the lymphocytes.

We observed the complete disappearance of eosinophils from the peripheral blood during the experiments in both the "hyperoxic" and control animals. /499

Most of the changes in the content of white blood cells can be attributed to the redistribution of leukocytes, more precisely, to their temporary deposition. The period of leukocyte circulation in the peripheral blood is known to be relatively short. The cells are quickly deposited in the capillary network of several organs, particularly the lungs, an organ closely connected with the external environment.

This fact warrants our special attention, for exposure to hyperoxia impairs the pulmonary circulation (hyperemia, stasis) with resultant edema and pulmonary inflammation (refs. 2 and 18). There may also be considerable deposition of lymphocytes (which are the most mobile formed elements of the blood in this respect) followed by a decrease in their number in the bloodstream.

We do not have as yet any direct indices of the function of lymphoid tissue in experimental animals, but it is quite likely that a high partial pressure of oxygen in the atmosphere also alters the course of lymphopoiesis.

After 6 days of exposure to hyperoxia, a sudden change seems to take /500 place in the form of an intensified flow of lymphocytes into the bloodstream, possibly the result of the toxic action of oxygen.

We also noted some changes in the granular leukocytes. For example, exposure to 60 and 90 percent oxygen brought about a slight transient neutrophilosis in the experimental animals with a shift to the left, suggesting a stimulation of myeloid hematopoiesis. By the 10th day of exposure to 60 percent oxygen and thereafter, a second increase was observed in the number of granulocytes. This can also be related to the oxygen intoxication manifested at these periods of time.

Some of the changes found in the blood morphology of the experimental animals were undoubtedly a manifestation of a nonspecific "stressor" reaction to environmental changes (placement in the chamber, noise of the regenerator, etc.). The increase in neutrophil content with a shift to the left and disappearance of the eosinophils are examples.

The same kind of changes also occurred in the control mice, although less distinctly. They were described by us in experiments in which the animals were kept for several days in a closed space with an ordinary oxygen content (ref. 4). These reactions obviously develop with the participation of certain neurohumoral mechanisms (ref. 12).

However, we must note the characteristic effect of hyperoxia on the white blood cells of mice, leukopenia, which results from a marked decrease in the lymphocyte content combined with brief neutrophilosis. This feature makes the effect of an atmosphere with increased oxygen concentration similar to the effect of ionizing radiation, suggesting that the two factors have a common mechanism of physiological action. In point of fact, many authors (refs. 15 and 19) ascribe a major role in the primary links of the radiobiological effect to the formation of peroxide radicals, which may occur during the action of oxygen on the organism (ref. 21).

This accounts for the protective function of several sulfur-containing compounds both in respect to the toxic effect of oxygen and in respect to radiation injury (ref. 8). It is no wonder, then, that the simultaneous or successive action of ionizing radiation and compressed oxygen sharply reduce the survival rate of mice. There are also references in the literature to the effect that oxygen may cause fractures of chromosomes analogous to those following irradiation. High concentrations of oxygen in the atmosphere have been shown to inhibit cell proliferation in a culture as a result of injury to the mechanism of multiplication (ref. 17).

Without deciding at this time the intimate mechanism of action of oxygen on the blood system, the foregoing data point to the possibility of hyperoxia exerting a genetic effect (of which the morphological composition of the blood may serve, to some extent, as an indicator). This effect is obviously /501 most dangerous when increased partial pressure of oxygen in the atmosphere is combined with ionizing radiation.

We conclude from our investigations that blood morphology is a sensitive indicator which should be used to evaluate the physiological effect of increased partial pressure of oxygen on the organism.

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ADAPTATION TO DIFFERENT FORMS OF HYPOXIA

COMMUNICATION 1. EFFECT OF GRADUAL AND SUDDEN HYPOXIA

V. A. Konstantinov

An oxygen deficiency is a very important problem in space medicine, /502 for it is just as difficult to provide man with oxygen during prolonged spaceflight as it is to keep him supplied with food. Spaceflight and planetary explorations will take place in an environment with total absence of oxygen. A variety of factors (according to the literature, more than 25) may produce oxygen deficiency. Since the body has almost no oxygen reserve (ref. 22), hypoxia may arise gradually or with lightning speed, depending on the emergency situation.

Analysis of the physiological changes resulting from decrease in the amount of oxygen in inhaled air was the object of our investigation. This report describes the results of a study of two forms of oxygen deficiency: (1) gradual decrease in the oxygen content of inhaled air (first form of hypoxia); (2) sudden decrease in the oxygen content of inhaled air to 5-7 percent (second form of hypoxia).

A gradual decrease in oxygen (first form of hypoxia) was brought about in the following ways:

(1) A closed respiratory system was created in fixed cats by means of a gas anesthesia apparatus and connected to a rubber bag containing a precisely measured amount of air (10 liters). Valves were used to circulate air in one direction through a case with a chemical agent to absorb the carbon dioxide released. Oxygen in the system was decreased according to animal demands.

(2) Oxygen was gradually decreased in the inhaled air of cats al- /503 lowed to move freely in a chamber (280 liters) through which nitrogen was passed.

(3) Experiments were performed on rats in hermetically sealed jars.

Respiratory standstill or appearance of agonal inhalations was an indication that the experiment was finished.

The oxygen content of inhaled air was suddenly decreased (second form of hypoxia) only in cats fixed on a bench. A tank with a prepared gas mixture containing 5-7 percent oxygen was connected to a tube inserted into the trachea.

Oxygen was supplied in all experiments after respiratory standstill. The animal resumed spontaneous breathing either at once or after a few artificial inhalations. This made it possible to reinduce an oxygen deficiency in the same animal and under the same conditions after 10-15 minutes, and to observe how it differed from the preceding deficiency.

The condition of the animal was judged from the following indices: arterial pressure; rate and depth of respiration; body temperature; reflex changes in arterial pressure in response to compression of the aortic artery and painful stimulation of the tibial nerve; change in oxygen content of the inhaled air; content of erythrocytes, leukocytes and hemoglobin; oxygen saturation of the blood. The results of the investigations were statistically processed. The principal findings, summarized in the table, show significant differences in the standard deviations.

In the first form of hypoxia (gradual decrease in oxygen content), distinct adaptation reactions appeared. Elevated arterial pressure persisted, increasing somewhat as the oxygen deficiency developed. The amplitude and frequency of respiratory movements increased. High arterial pressure following compression of the carotid artery and pain persisted a long time. The blood oxygen level dropped slowly. The mean duration of hypoxia was 61 minutes, at which time the mean oxygen content of the inhaled air was 6.2 percent. Rectal temperature dropped 0.8° (table 1).

The initial indices of arterial pressure and respiratory rate before the second exposure to hypoxia were somewhat lower than before the first exposure. However, the second exposure showed that the adaptation mechanisms were not only preserved, but were even more pronounced, because arterial pressure, respiratory rate and blood oxygen content remained for some time at a high level. Reflex changes in arterial pressure following compression of the carotid artery /505 and application of pain also exceeded the original level for a while. The animals required less oxygen per unit of time than during the previous exposure, because with an equal initial amount of oxygen (identical volume of the hermetically sealed system), it remained longer in the system after a second exposure for the same length of time (45 min). Respiratory standstill generally occurred only with 4.3 percent oxygen in the inhaled air. This resulted in the mean duration of the repeated hypoxia lengthening to 98 min, i.e., an increase of 60 percent (fig. 1).

The duration of the repeated hypoxia (table 2, fig. 2) lengthened in different versions of the experiments and from animal to animal (ref. 11). Ye. A. Kovalenko (ref. 10) observed the phenomenon in dogs.

With the second form of hypoxia (sudden change to breathing a gas mixture containing 5-7 percent oxygen), the picture was different. After transient motor restlessness, respiratory disorders developed and blood pressure fell rapidly. Reflex rises in arterial pressure following compression of the carotid artery and application of pain scarcely exceeded the original /507 level.

TABLE 1. RESULTS OF EXPERIMENTS WITH GRADUAL DECREASE IN OXYGEN CONTENT OF INHALED AIR (FIRST FORM OF HYPOXIA) AND WITH SUDDEN ONSET OF HYPOXIA (SECOND FORM OF HYPOXIA).

Experimental conditions	No. of investigations	Gradual hypoxia		No. of investigations	Sudden hypoxia	
		First	Repeated		First	Repeated
In mice in hermetically sealed place--duration of hypoxia, min*	7	13 ± 2	28 ± 3	-	-	-
In rats in hermetically sealed place--duration of hypoxia, min	8	45 ± 4	54 ± 5	-	-	-
In cats moving freely in chamber--duration of hypoxia, min	5	86 ± 9	108 ± 11	-	-	-
Oxygen content of inhaled air (in %) at end of hypoxia	5	3.3 ± 0.2	2.8 ± 0.3	-	-	-
In fixed cats--duration of hypoxia, min	14	61 ± 5	96 ± 6	12	20 ± 3	11 ± 1
Body temperature (in °C)						
at start of experiment	11	37.2 ± 0.8	36.3 ± 0.6	12	36.9 ± 0.2	36.0 ± 0.3
at end of experiment	11	36.4 ± 0.7	35.4 ± 0.7	12	36.0 ± 0.3	35.6 ± 0.2
Oxygen content (in %) of inhaled air						
after 45 min	8	14.3 ± 0.5	19.3 ± 0.4	-	-	-
at end of hypoxia	14	6.2 ± 0.1	4.3 ± 0.2	-	-	-
Original content (millions in 1 mm^3) of erythrocytes	8	8.46 ± 0.1	8.17 ± 0.1	12	8.81 ± 0.4	9.01 ± 0.3
at end of hypoxia	8	8.17 ± 0.1	7.42 ± 0.2	12	9.01 ± 0.3	9.11 ± 0.4

*According to published data (ref. 11).

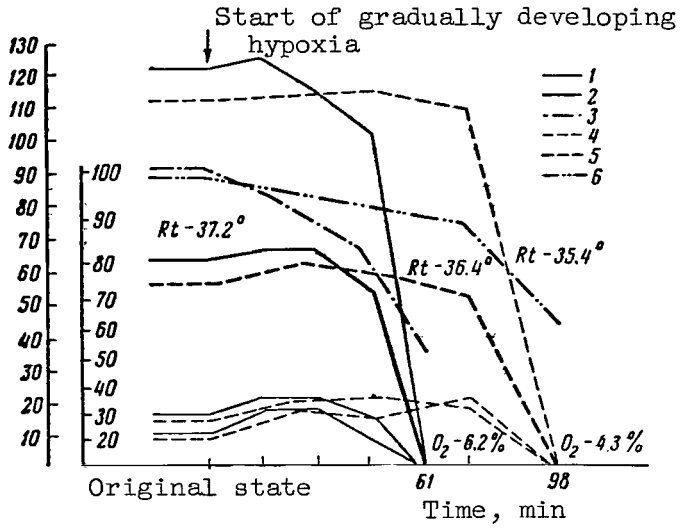

Figure 1. Graphic representation of mean results of experiments with gradually developing hypoxia. First hypoxia: 1, arterial pressure; 2, respiration; 3, HbO_2 content. Second hypoxia: 4, arterial pressure; 5, respiration; 6, HbO_2 content.

Lower curves, elevation of arterial pressure in mm Hg following compression of carotid artery and application of pain.

TABLE 2. MEAN RESULTS OF EXPERIMENTS WITH GRADUALLY DEVELOPING HYPOXIA IN DIFFERENT ANIMALS.

Experimental conditions	No. of experiments	Hypoxia	
		First	Repeated
In mice in hermetically sealed place*-- duration of hypoxia, min	7	13 ± 2	28 ± 3
In rats in hermetically sealed place-- duration of exposure, min	8	45 ± 4	54 ± 5
Change in body temperature with original temperature of 37.7 ± 0.9°	8	34.4 ± 0.4	31.9 ± 1.0
In cats moving freely in chamber			
duration of hypoxia, min	5	86 ± 9	108 ± 11
oxygen content at end of hypoxia, %	5	3.3 ± 0.2	2.8 ± 0.3

*According to published data (ref. 11).

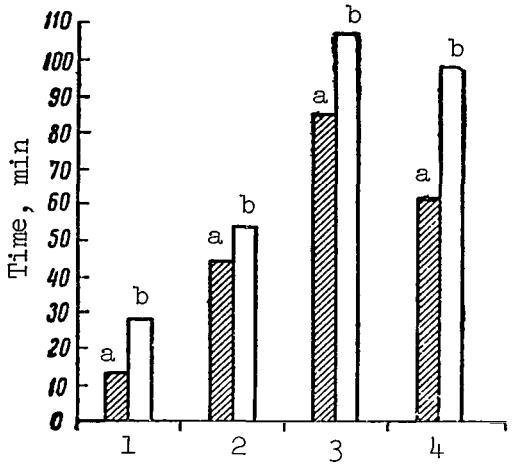

Figure 2. Different versions of experiments with gradually developing hypoxia. a, mean duration of first hypoxia; b, mean duration of repeated hypoxia. 1, Mice in jar; 2, rats in jar; 3, cats in chamber; 4, cats with closed respiratory system.

The repeated hypoxia (unlike the first form) continued on the average almost half as long and it was accompanied by an even more rapid drop in arterial pressure and respiratory disturbances. In most of the experiments these disturbances were quite characteristic and consisted in the appearance of marked wavelike fluctuations of arterial pressure and periodic respiration (fig. 3a and b). Reflex changes in arterial pressure following compression of the carotid artery and application of pain began to decrease immediately. The blood oxygen level fell more quickly than during the first hypoxia (fig. 4).

In four experiments, sudden inhalation of a gas mixture containing about 8 percent oxygen for an hour did not result in respiratory standstill the first time. However, a second exposure to the same mixture produced marked functional disturbances within 15-25 min and killed the animals.

Many investigators have concerned themselves with the problem of adaptation to hypoxia. Z. I. Barbashova (refs. 3 and 4) reviewed the extensive material in several articles. Adaptation to an oxygen deficiency is a complex and multifaceted process involving various body systems and tissues. Compensatory reactions of varying intensity, nature and predominance of a particular defense mechanism arise, depending on the degree of hypoxia.

Most authors endeavored to induce hypoxia in animals by means of comparatively sluggish adaptation mechanisms--by daily prolonged training exercises and placing the animals in an environment with a low oxygen content (refs. 3, 5, 8, 15, 16 and 20). During the training exercises the rate of change in oxygen content of the inhaled air (i.e., rate of "rise") was always comparatively high (no less than 1 km/min), for it was thought that the lower the oxygen content of the inhaled air, the more rapid were the manifestations of the defense reactions and, consequently, the development of adaptation.

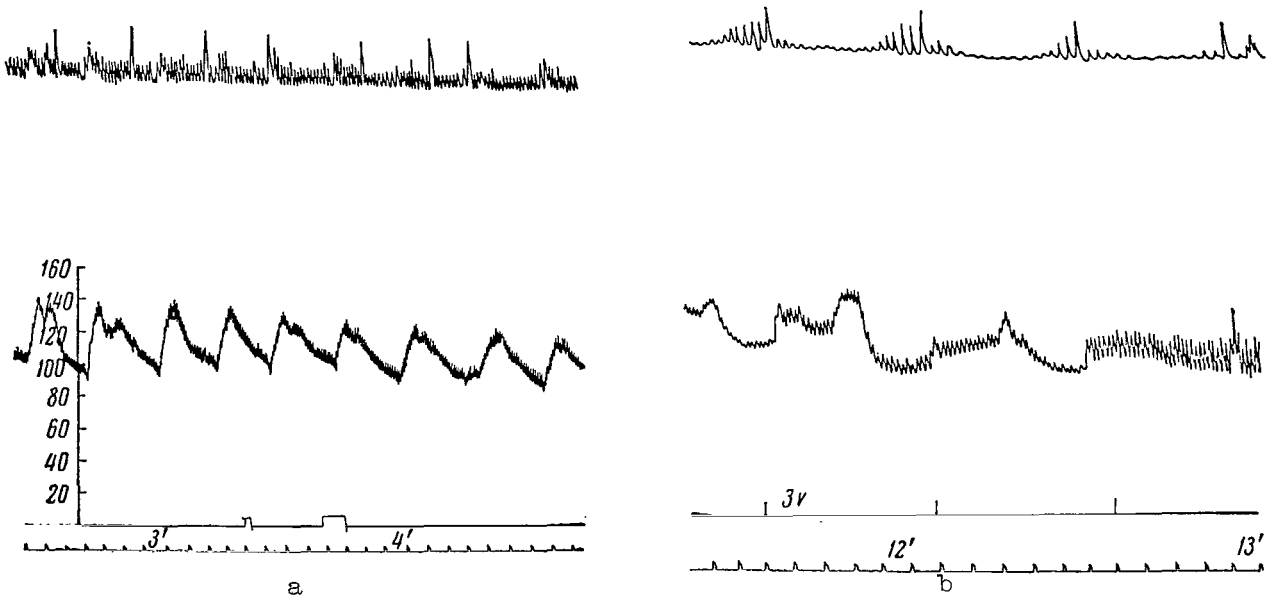

Figure 3. Course of sudden hypoxia. Characteristic disturbances in blood pressure (a) and respiration (b) during repeated hypoxia.

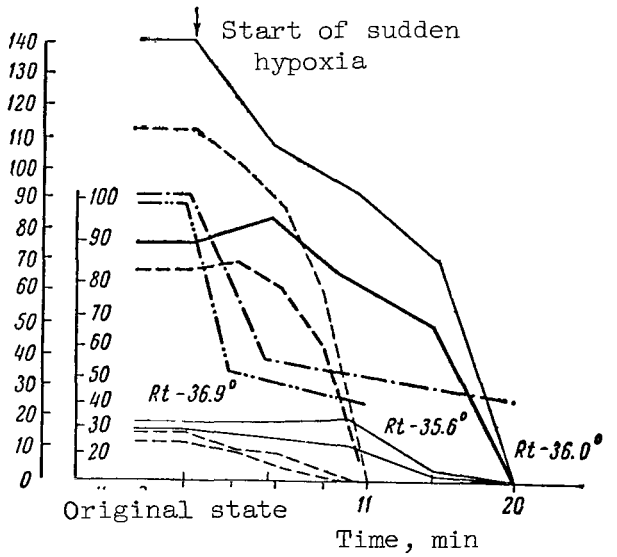

Figure 4. Graphic representation of mean results of experiments with sudden hypoxia. Symbols same as in figure 1.

The method whereby the "ceiling of the rise" rose daily (with rapid attainment of the given height being preserved) or when periodic halts were made during the rise was therefore considered the "slow rise" method. L. A. Podsosov calls the periodic halts "adaptation platforms."

Our experiments show that the rate of change in the oxygen content of inhaled air is an important factor. This is confirmed by various physiological characteristics and by the residual effects of the different forms of hypoxia which we studied. Gradual hypoxia resulted in decreased sensitivity to a minimum amount of oxygen in inhaled air. The second exposure to this form /509 of hypoxia was longer than the first. Of major significance were the increase in activity of the adaptation mechanisms in the "fight for oxygen" and the decreased oxygen requirement of the tissues.

Many authors (refs. 1, 2, 9, 11, 13, 18 and 19) showed that oxygen consumption decreases during hypoxia, with a further decrease taking place during a second exposure.

With sudden hypoxia, on the other hand, different functional changes took place, resulting in increased sensitivity to an oxygen deficiency, for during a second exposure to the same hypoxic mixture the animal's breathing stopped sooner than during the first.

Our experiments showed that sensitivity to hypoxia may decrease because of the activity of sluggish compensation mechanisms. It may also result from the activity of certain emergency defense mechanisms which are activated as soon as the body experiences a deficiency of oxygen.

The increased duration of the second hypoxia seems to be unrelated /510 to changes in body temperature. There is no direct connection between the lowering of rectal temperature and the duration of oxygen deficiency. Moreover, with sudden hypoxia the body temperature dropped by almost the same amount, but in none of the 12 experiments did it result in lengthening the second hypoxia.

The possibility of increasing resistance to an oxygen deficiency in a short time by repeated gradual hypoxia during spaceflight is quite tempting. Adaptation may occur and thus permit some saving on the amount of oxygen carried on board the spacecraft. Moreover, adaptation to hypoxia may increase resistance to other pathogenic factors as well. N. N. Sirotonin (ref. 17) was the first to show that adaptation to hypoxia has a favorable effect on the course of some mental illnesses. It was subsequently found to mitigate the course of such processes as deep hypothermia, pneumonia (ref. 14), burn disease (ref. 21), prussic acid poisoning (ref. 4), radial acceleration (ref. 16), radiation sickness (ref. 6) and oxygen poisoning (ref. 9).

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ADAPTATION TO DIFFERENT FORMS OF HYPOXIA

COMMUNICATION 2. CHARACTERISTICS OF THE COURSE OF EXTREME HYPOXIA

V. A. Konstantinov

One of the most significant types of oxygen deficiency is the /512
hypoxia that develops in a very brief period of time, e.g., at high altitudes and especially during spaceflight in conjunction with "explosive decompression" (refs. 1, 3, 4 and 6). In the literature, however, attention is focused mainly on changes in barometric pressure and on the effects of hypoxia combined with them (refs. 2, 5, 7, 8, 9 and 10). It should be remembered that a drop in barometric pressure has pathogenic effects (pain in the frontal and maxillary sinuses and middle ear, strong interoceptive stimuli from the distended intestine and other hollow organs, increase in intrathoracic, intrapleural and spinal fluid pressure, "boiling" of the blood and other fluids). All these phenomena mask changes resulting from an oxygen deficiency.

The purpose of our investigation was to study superacute or "extreme" oxygen deficiency in a "pure" form (without barotrauma) that develops in several tenths of a second.

A total of 165 experiments were performed on 35 cats. A rubber tube was inserted into the trachea of etherized cats, after which the animals were anesthetized with nitrous oxide. The usual preparations were made for recording arterial pressure, respiration and stimulation of the tibial nerve.

In the course of the experiment the animals were suddenly given pure nitrogen under normal pressure. After respiratory standstill, the respiratory system was immediately filled with oxygen and artificial respiration was started by rhythmic compression of the lungs.

The interval between supplying nitrogen and starting artificial /513
respiration was regarded as the duration of hypoxia.

The results of the experiments showed that hypoxia set in very quickly, in no more than 4 minutes. Motor excitation developed immediately with elevation of arterial pressure, deepening of respiration and sharp drop in the blood oxygen level. Reflex changes in arterial pressure in response to application of pain disappeared quickly.

A characteristic of this "extreme" hypoxia was the fact that repeated oxygen deficiency occurred in each animal in a very similar fashion. The first extreme hypoxia lasted 1.5-3 min and 20 sec. These experiments were intentionally performed with differences in the initial rectal temperature--from 37.7-32.1°C. Nevertheless, it was impossible to detect any correlation between the duration of hypoxia and body temperature.

Repetition of hypoxia 2-14 times in succession under the same conditions failed to have any effect on its duration. For a short period of hypoxia body temperature scarcely changed, but prolonged fixation of the cats to the bench lowered body temperature by 1.9-2.4°C. However, this failed to change the duration of repeated hypoxia.

Repeated extreme hypoxia was carried out at different intervals after the restoration of spontaneous breathing (from 5 min to 2.5 hours). These experiments are represented graphically in figure 1, which shows that the duration of extreme hypoxia remains the same in the same animal, despite frequent /514 repetitions.

The noteworthy thing about these experiments is the striking similarity in the course of oxygen deficiency. This was particularly evident in the experiments in which only the respiratory movements were recorded, when the surgical trauma (to record blood pressure) had no effect on the animal's condition (fig. 2). The figure shows that even an occasional deeper inhalation or change in amplitude of respiration appeared after precisely the same number of seconds as in the preceding hypoxia. This phenomenon also occurred in the restoration period.

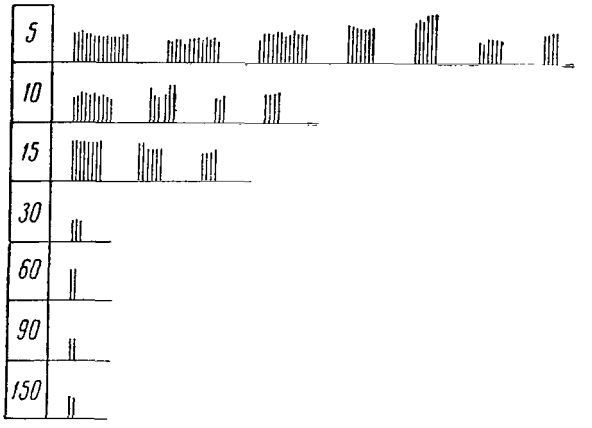

Figure 1. Graphical representation of duration of repeated extreme hypoxia in same animals. Vertical lines, duration of extreme hypoxia (in sec). Each series of lines pertains to experiment on 1 animal. Figures to left indicate intervals between repeated exposures to hypoxia (in min).

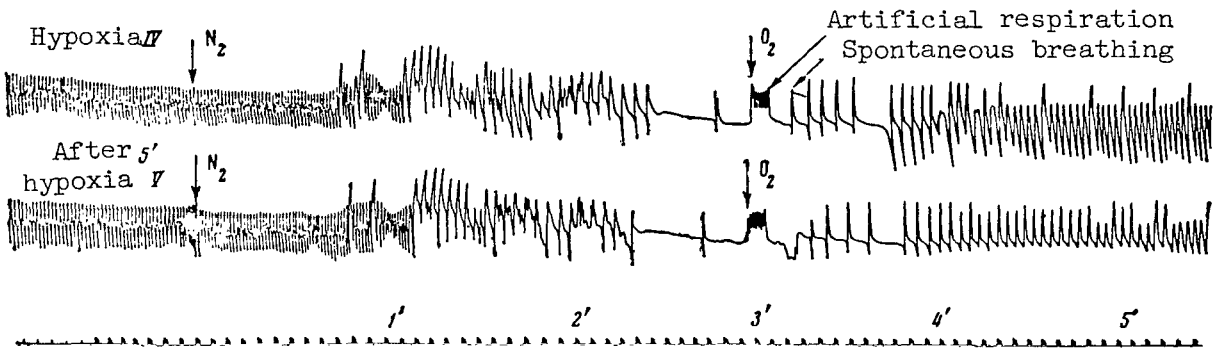

Figure 2. Recording of respiratory movements in cat repeatedly exposed to extreme hypoxia. Lower line, time mark (5 sec).

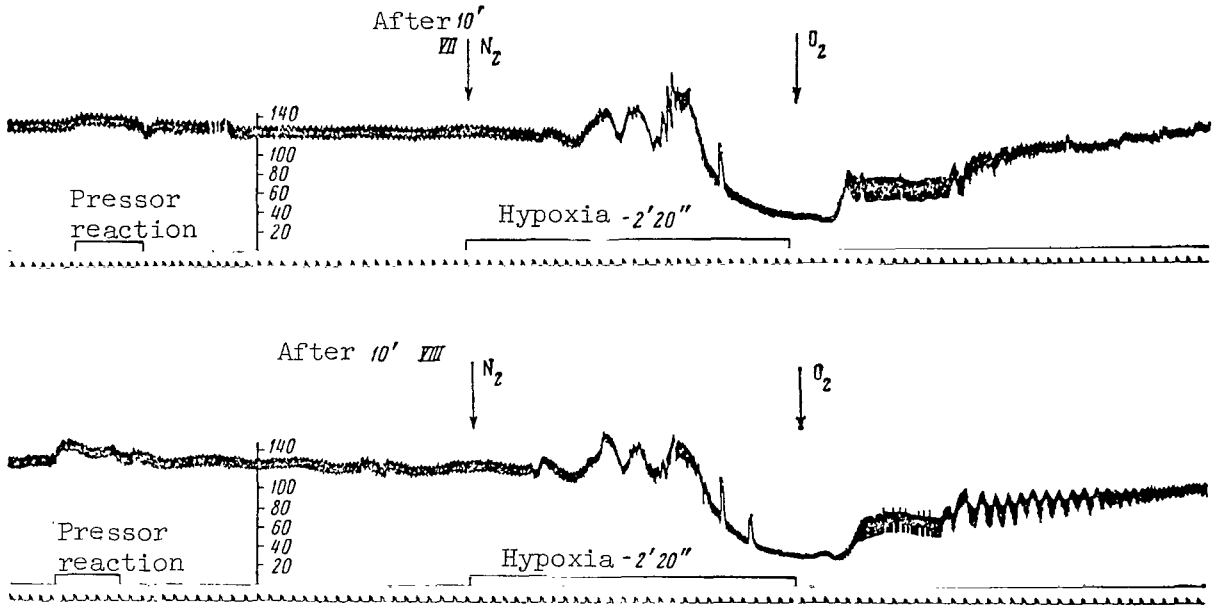

Figure 3. Recording of arterial pressure in cat repeatedly exposed to extreme hypoxia. Lower line, time mark (5 sec).

The changes in arterial pressure (fig. 3) and oxygen saturation of the blood (fig. 4) were also similar.

Substitution of nitrous oxide for inert nitrogen also failed to change either the nature or the duration of extreme hypoxia.

Analysis of the blood for content of erythrocytes, leukocytes and hemoglobin revealed no significant changes either after single or repeated exposures

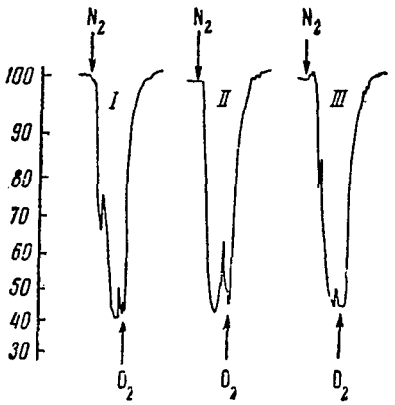

Figure 4. Changes in oxygen saturation of blood (in arbitrary units) in cat repeatedly exposed to extreme hypoxia.

TABLE 1. HEMATOLOGICAL INDICES AFTER REPEATED SUPERACUTE HYPOXIA (MEAN OF 7 EXPERIMENTS).

Content, mm^3	Original data	After first hypoxia	After repeated exposures
Erythrocytes, millions	8.4 ± 0.49	8.5 ± 2.3	8.3 ± 0.6
Leukocytes, thousands	16.7 ± 5.4	17.8 ± 2.3	22.0 ± 4.4
Hemoglobin, g %	15.6 ± 0.7	16.1 ± 0.7	15.8 ± 1.4

to hypoxia (table 1). There was only an increase in the leukocyte count after several exposures.

The experiments showed that this form of hypoxia is different from /515 the other forms.

Its main feature, the similarity in course of the oxygen deficiency, may be explained as follows. A closed respiratory system is very quickly filled with nitrogen. Therefore, the vital activity of the organism depends solely on the rapidity with which the existing oxygen reserves in the blood and tissues are exhausted.

However, the oxygen capacity of the blood at a normal barometric pressure is fairly constant. Hence, the reason for the uniformity of the picture of hypoxia is that it arises each time with the original amount of oxygen in the blood and tissues being identical.

This is confirmed by the fact that the duration of hypoxia can be shortened even more if it is induced when the blood oxygen is at a lower level. Under the control of a flow-type oxyhemograph, the animals breathed a hypoxic mixture as

TABLE 2. CHANGE IN DURATION OF SUPERACUTE HYPOXIA IN RELATION TO ORIGINAL OXYGEN CONTENT OF THE BLOOD.

No. of experiments	Original oxygen content, a.u.*	Duration of hypoxia, min	Oxygen content, in a.u., at respiratory standstill
20	99 ± 2.8	2.7 ± 0.15	42 ± 2.0
12	86 ± 2.0	1.4 ± 0.14	42 ± 1.7

*a.u. = arbitrary units

long as the oxygen content of the blood did not fall by more than 12-16% below the original level. With this oxygen content of the blood, the duration of a repeated hypoxia did not average 2.7 min, but was almost half as long--1.4 min (table 2). It is curious to note that the minimum oxygen level of the blood at which respiratory standstill set in did not change.

Extreme hypoxia produced a distinct increase in the amplitude and /517
rate of respiration and elevation of arterial pressure. However, these reactions, directed mainly at ensuring a higher rate of delivery of oxygen to the tissues, were largely futile, because the organism was immediately confronted with a total absence of oxygen in the inhaled air.

Our experiments indicate that the duration of extreme hypoxia was related both to physiological factors (compensatory reactions) and to the biological properties of hemoglobin and other tissues capable of retaining and releasing a definite amount of oxygen.

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EFFECT ON CERTAIN PHYSIOLOGICAL FUNCTIONS OF PROLONGED EXPOSURE OF ANIMALS TO CONDITIONS OF AN OXYGEN-ENRICHED AIR MEDIUM

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According to the literature, maintenance in a cosmonaut's cabin of a /518 gaseous environment corresponding to the Earth's atmosphere fails to satisfy the requirements of prolonged spaceflight.

At the present time there is no consensus concerning the permissible level of oxygen content in the atmosphere of a spacecraft.

Most authors favor the maintenance of partial pressure of oxygen in a cosmonaut's cabin comparable to atmospheric at both normal and low barometric pressures ranging from 390-405 mm Hg (refs. 24, 51, 54, 56), recommending the use of gas mixtures with increased partial pressure of oxygen at maximal permissible physiological level (400-425 mm Hg) and low barometric pressure because of partial or complete exclusion of nitrogen.

A number of authors (refs. 36, 49, 50) believe that a cosmonaut in a spacesuit should breathe pure oxygen at a pressure of 0.35 atmosphere; others (refs. 31, 45) favor for the cabin an oxygen-enriched atmosphere even with pressures close to one atmosphere.

The maintenance of oxygen in the atmosphere of a spacecraft cabin at a high level or filling with oxygen the space under a spacesuit is based on the following reasons: the first is to prevent the possible rapid onset of hypoxia or aeroembolism in case of rupture of the cabin's hermetic sealing (refs. 1, 44). It is also important to retain a high concentration of oxygen as an emergency reserve in case of accident (ref. 31). /519

A number of statements are to be found concerning the favorable influence of high concentrations of oxygen on a number of physiological functions and increasing resistance to various stress conditions which frequently occur in spaceflights: overloads, sharp changes in temperature, high concentrations of carbon dioxide, drop in oxygen content, accumulation of carbon monoxide, vestibular disturbances, etc. (refs. 2, 19, 20, 43). The majority of these factors are obviously temporary, requiring but brief recourse to breathing pure oxygen or an oxygen-enriched gas mixture.

In this connection, Ye. M. Sazonova's work (ref. 23) is of interest; it refers to intensification of myocardial contractility on breathing a gas mixture containing 60 percent oxygen. The natural load of a heart is known to decrease under conditions of weightlessness; in spaceflight this could be a detrimental factor. It may be assumed that the breathing of oxygen-enriched gas mixtures normalizes to a considerable degree and the decreased afferent impulses from the proprioceptors and interoceptors that occur during weightlessness because hyperoxia intensifies impulses from various receptor zones and increases the tones of the cardiovascular system (refs. 3, 14, 42).

Data are also available concerning the favorable effect of oxygen on the activity of the central nervous system (ref. 16) and on increasing work capacity (ref. 46). Of special significance is the normalizing effect of oxygen on the level of metabolic processes, lowering of reactivity and other functions. These, in our opinion, may act as positive factors in certain adverse situations that can take place in spaceflight (refs. 11, 26, 39).

On the other hand, protracted breathing of oxygen-enriched mixtures exerts a toxic action on the organism. The majority of authors allow a safe limit for breathing of oxygen under pressure by man of 400-425 mm Hg at both normal and low barometric pressure; this corresponds to 52-56 percent oxygen content at sea level. But this level is only relative and debatable. Studies in recent years on people and animals showed that breathing of oxygen at high altitudes caused atelectasis and other pulmonary disorders (refs. 38, 47).

However, all these investigations lack an exhaustive study of a number of physiological functions. The authors usually based their opinion of safe periods of breathing oxygen on the survival rate of animals and on the absence of /520 manifestly pathological changes in the lungs and other organs.

The literature contains statements on the impossibility of survival in an atmosphere lacking in nitrogen (ref. 10), but there is as yet no experimental confirmation of this thesis. No prolonged experimental exposure of the human or animal organism to an atmosphere enriched with oxygen has as yet been carried out. Hardly any data exist on the possibility of the organism adaptation to high partial pressure of oxygen.

For the above reasons, scientific determination of optimal concentrations of gaseous components for the atmospheres of spacecraft scheduled for long flights is an important problem, the solution of which calls for a thorough and meticulous analysis of the mechanism of influence of modified gaseous environments and the characteristics of the organism's adaptative reactions to them. The latter question is of special interest because creatures living on the surface of our planet are not exposed under natural conditions to a high oxygen and a low nitrogen content. Evidently, adaptation to such conditions of existence will be more difficult and take longer than, for example, adaptation to oxygen insufficiency.

The objective of our research was to study the character of changes in certain fundamental functions of the organism in relation to the duration of exposure to a gaseous mixture corresponding to the top safe limit of oxygen content (400-425 mm Hg) and to abrupt change in the composition of the gaseous environment.

Experiments were carried out on male white mice two to three months old in a closed system filled with air or a gaseous mixture containing 63 percent oxygen for a period of 0.5, 1.5, 5.0, 6.0, 8.0, and 10.0 days.

The animals were divided into three groups: the first, the experimental group, was kept in a closed system containing oxygen-enriched air; the second and third groups, the controls, breathed ordinary air, one in a closed medium, the other breathing room air. All the groups were otherwise maintained under identical conditions.

The experiments were carried out in hermetic chambers, 7 and 400 liters in size, similar to Shaternikov's respiration apparatus. Chambers for the first group had an environment containing 62-64 percent oxygen; the control chambers had an air environment with pressures close to one atm. Carbon dioxide, moisture, and volatile substances released by the animals were removed with the help of special absorbing agents and filters. The supplying of oxygen free from nitrogen and measurement of its consumption in a small chamber were achieved with special apparatus (ref. 7).

Experimental animals in the large chamber were kept 10-12 together in /521 small plexiglass cages. The walls of the cages had numerous openings for changing air and special feed boxes and water receptacles to provide for the entire length of an experiment. In case of need, they could be replaced without disturbing the chamber's gaseous composition. The system included cartridges with agents to absorb moisture (silica gel, calcium chloride) and carbon dioxide (chemical absorbents) and also a filter for certain volatile admixtures (activated charcoal). The air leaving the chamber had some of its excess moisture removed by being passed through a coil submerged in cold water. A blower circulated the air through the system at 20-40 liters per minute (fig. 1). Oxygen was automatically supplied as consumed by a gas meter from a cylinder. Any slight drop in the chamber's pressure (2-3 mm Hg) was automatically corrected.

During the course of an experiment, a Haldane gas analyzer systematically analyzed the air in the special and control chambers. The carbon dioxide content was determined in a dynamic optico-acoustic gas analyzer of the OA-2209 system.

Twelve mice were put into the small chamber at one time for experiments not exceeding 5 days. About 100 were put into the large chamber with the experiment extended to 10 days.

The condition, behavior, rate of respiration, and gas exchange were studied during the time the animals were in the closed system. Upon termination of the experiment, the following physiological indices were studied: condition of the central nervous system, level of gas exchange, morphological composition of the peripheral blood, and resistance to acute oxygen deprival. Active selection of an optimal gas environment by the animals was studied with the help of special apparatus. Observations were carried out on weight dynamics and the condition of the lungs. In some of the experiments the thyroid gland and other organs were examined histologically.

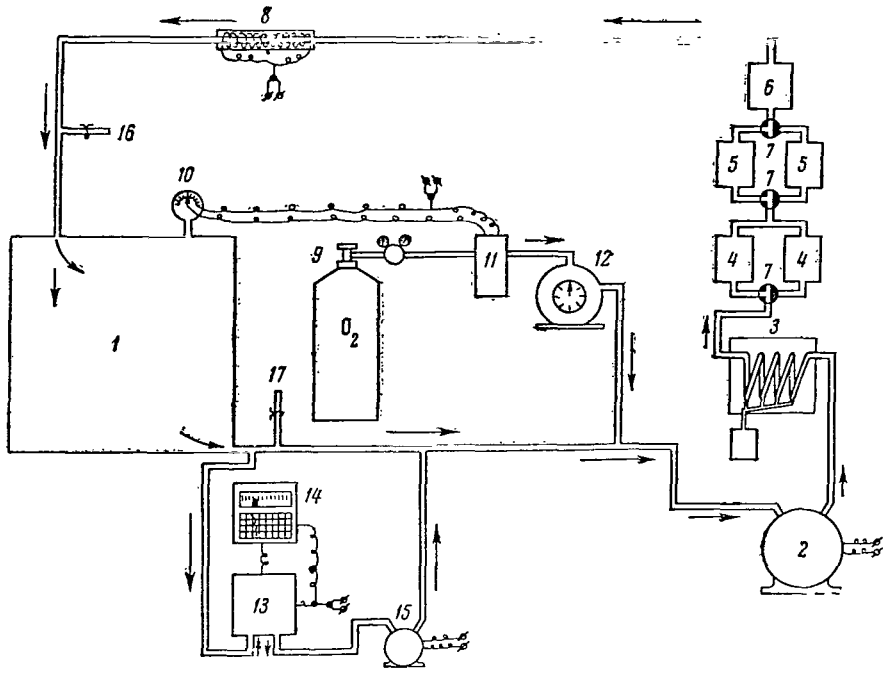

Figure 1. Diagram of a closed system for regenerating air and determining gas exchange in animals in long-term experiments: 1, chamber containing the experimental animals; 2, blower; 3, condenser for condensing moisture; 4, 5, 6, filters with chemical absorbent, silica gel, and activated charcoal; 7, three-way cock for switching air during filter replacement; 8, air heater; 9, oxygen cylinder; 10, manometer with terminals; 11, automatic device for supplying oxygen upon a drop in the system's pressure; 12, meter for measuring oxygen supply; 13, 14, 15, intake chamber, indicating and recording instrument, activator for input of the automatic gas analyzer; 16, 17, pipes for withdrawal of air samples from the system.

The condition of the central nervous system was evaluated from unconditioned reflexes (the time the mice required to traverse a maze, the number of errors, the intensity of the current used as a stimulus). An alternating current of 7-20 volts was passed through a mesh used as the floor for the maze. Record was kept of the lapse of time from the moment the current was turned on to the moment the animal reached an insulated platform. Both the experimental and control mice were tested daily in this way for 12 days after termination of the experiment.

The resistance of mice to hypoxia was studied in a 150-liter chamber in which hypoxia was induced by replacing air with nitrogen which was fed in from a cylinder at the rate of 10 liters per minute. At the end of 30-40 minutes, the gas mixture in the chamber contained 2-3 percent oxygen.

The condition of the lungs was determined by noting the ratio of their weight to the weight of the body. The coefficient of this ratio reflected the degree of pulmonary edema. This method proved its worth in studying the effect of oxygen on pulmonary tissue (ref. 32). The lungs were weighed on a torsion scale with an accuracy of up to one gram.

The respiratory rate was studied during the day throughout the entire experiment. The figures were statistically processed by the Student-Fisher's method.

The respiratory rhythm of the mice kept in the hyperoxic environment /523 was perceptibly slower than that of the control animals. Respiration started to slow during the first day of the experiment and progressed on the second and third days. From then on up to the 10th day of the experiment, the respiratory rate remained relatively constant with a relatively low level of fluctuation. When the experiment was over the animals were returned to an air environment, respiration accelerated but did not reach the rates of the control animals (fig. 2).

These changes were statistically significant ($P < 0.05$).

The hyperoxic environment also influenced the level of gas exchange in the mice. Oxygen consumption in the experimental group was slightly higher than in the control (the difference was statistically insignificant). The biggest difference was observed at night, when the increase in metabolism that is part of their daily rhythm characteristically takes place.

By the end of the 5th day of the mice's exposure to a hyperoxic environment, their gas exchange showed a tendency to decrease. Furthermore, when they were subsequently given air to breathe, the gas exchange level dropped perceptibly, becoming lower than that of the control animals. Replacement of the hyperoxic environment with air or a hypoxic environment resulted in a noticeable temporary decrease in gas exchange. Upon replacement of the air with a hyperoxic mixture, there was a temporary increase in gas exchange.

Histological examination of the thyroid gland of the mice in these experiments showed lowered functioning of this gland following exposure to a hyperoxic environment. This was borne out by decreased height of the follicular epithelium, by overfilling of the follicles with colloids, and by other morphological changes.

The difference in the duration of the animals' exposure to a gaseous environment containing 63 percent oxygen affected in varying degrees the weight dynamics of the experimental animals. A definite pattern became apparent in the weight increase of the "oxygen" mice which depended on the duration of their exposure to an oxygen-enriched gaseous environment. The weight of the experimental mice was somewhat greater than that of the control animals during the first 6 days of the experiment. Beginning with the 8th day of the experiment, the weight of the "oxygen" mice began to drop and almost reached the weight of the control group. On the 10th or last day of the experiment and on the third day following transfer

to breathing oxygen, their weight continued to decrease. It lagged 13.5 percent behind that of the control group. There was subsequently a tendency for the weight to increase, but by the 7th and 10th days it had not yet reached the value of the control group (fig. 3).

Appreciable changes also occurred in the other physiological indices. For example, specific changes took place in the blood picture: decrease in hemoglobin and in the number of erythrocytes, reticulocytes, and lymphocytes. These /524 changes were intensified after 5-6 days of exposure to a hyperoxic environment.

Certain changes took place in the neuroreflex reactions of the animals. The time it took the mice to traverse the maze after 6 and 10 days' exposure to a hyperoxic environment was greater than for control mice. A very distinct and persistent increase in traverse time was observed after the 6-day experiment primarily because of entrances into blind alleys of the maze. It was pronounced throughout almost all the tests following transfer of the animals to breathing air. Traverse time equalled that of the control group only on the 12th day. In the 10-day experiment, this pattern was observed only during the first two days following termination of the experiment. Traverse time thereupon became slightly shorter than that of the control group. This difference disappeared around the 19th day (fig. 4). It should be pointed out that the increase in traverse time was statistically significant only for the first two days of the tests. Approximately the same pattern was noted in the number of blind alleys entered by the mice. In these experiments, a decrease in excitability of the central nervous system was noted after 6 days in the hyperoxic environment as manifested by having to increase the current to induce the mice to traverse the maze. No such pattern was noted in the 10-day experiment, where the intensity of the current remained practically identical for both the experimental and control mice.

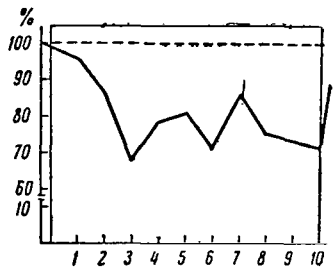

Figure 2. Dynamics of rate of respiratory movements in the animals during exposure to an environment containing 63 percent oxygen (in percent of the control).

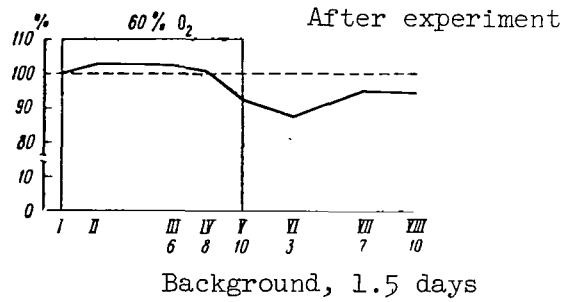

Figure 3. Changes in weight of the animals during their exposure to an environment containing 60 percent oxygen and thereafter (in percent of change in the control); I, before the experiment; II, 1.5 days after start of the experiment; III, after 6 days; IV, after 8 days; V, after 10 days; VI, 3 days after the experiment ended; VII, 7 days after; VIII, 10 days after.

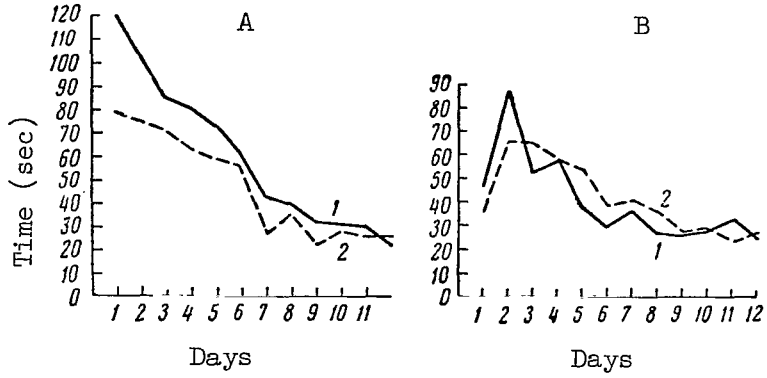

Figure 4. Dynamics of traverse time (in seconds) by the animals through a maze following electrical stimulation; A, after 6 days; B, after 10 days of exposure to an environment containing 63 percent oxygen; 1, experimental group; 2, control group.

Our hypoxia test disclosed an increase in the resistance of the experimental mice to hypoxemia. Their survival rate between the 6th and 10th days of the experiment was slightly higher than that of the control animals. By the third day after the transfer to breathing air, the survival rate was the same as that of the control group (fig. 5). /525

The effect of oxygen on the lungs became noticeable on the 6th day of the experiment and it became more pronounced around the 10th day. The coefficient reflecting the ratio of the weight of the lungs to body weight increased almost by 13 percent over the control group ($P < 0.05$). Upon transfer of the animals to breathing air, the coefficient began to decrease, becoming the same as that of the control group 10 days after termination of the experiment (fig. 6).

Special experiments showed that if the mice were exposed for a day and a half to a gas mixture containing 60 percent oxygen, the relative weight of the lungs remained the same. However, if the mixture contained 90 percent oxygen, a distinct increase in the weight of the lungs would take place. The same thing happened if they were exposed for a brief period of one and a half hours to oxygen under increased pressure (fig. 7). It should be noted that the mice were sacrificed and the lungs weighed in the latter case after the same period of time as in the preceding experiments, namely, 36 hours after the oxygen was first applied.

The resultant data showed that the oxygen-enriched air had a definite effect on a number of physiological indices. The changes may be considered both as a general reaction to a modified external and internal gaseous environment and as a specific reaction. /526

The distinct slowing of respiration in the animals breathing a hyperoxic mixture agrees with the data found in the literature (refs. 33, 34, 55). The decrease in the respiration rate is evidently a specific compensatory reaction

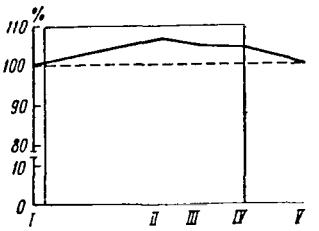

Figure 5. Resistance of the animals to oxygen starvation during their exposure to an environment containing 63 percent oxygen and thereafter (survival time under hypoxic conditions, in percent of the control); I, before the experiment; II, 6 days after start of the experiment; III, 8 days; IV, 10 days; V, 3 days after termination of the experiment.

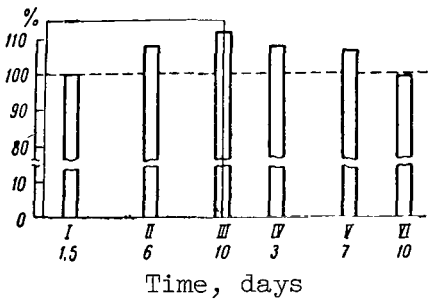

Figure 6. Relative weight of the animals' lungs during exposure to an environment containing 60 percent oxygen and thereafter (in percent of control); I, 1.5 days after the experiment began; II, 6 days; III, 10 days; IV, 3 days after termination of the experiment; V, 7 days; VI, 10 days.

aimed at decreasing the entry of oxygen into the organism. This reaction is indicative of a decrease in excitability of the respiratory center (ref. 48) and apparently excitability of the other nerve centers.

The last supposition is confirmed by the lowered motor activity of the mice, by increased maze traverse time, by an increase in the number of errors, and by the necessity for stepping up the electric current to make the runs possible with respect to the "oxygen" mice following termination of the 6-day experiment. At the end of the 10-day experiment, only a slight increase in traverse time and in the number of errors was observed during the first two days that followed. The intensity of the electric current was no different from that of the control group. The foregoing indicates that changes in the central nervous system--decreased

excitability and impaired differentiation--were more marked in the "oxygen" mice at the end of the 6-day experiment than at the end of the 10-day experiment. In the mice that had participated in the 10-day experiment, these changes /527
were less pronounced despite preservation of the excitability of the nerve centers equal to the control animals. This may have been due to adaptation of the animals to the oxygen by the 10th day of the experiment.

Our data on the gas preferendum confirm the disturbance of certain aspects of nerve center activity and their decreased excitability. The experimental mice differentiated to a lesser degree the high level of oxygen in their environment; they preferred deviations from the average level of oxygen in the direction of an increase. A decrease in the ability to distinguish differences in the oxygen content of gaseous environments may be explained by the adaptation of the animals' chemical receptors to oxygen. This is borne out by the data which show the importance of chemoreceptors in the reactions to the breathing of oxygen-enriched mixtures (refs. 14, 15, 18, 42). However, one cannot exclude the possibility that the processes contributing to impairment of differentiation in the central nervous system may play a part in reducing the animals' capacity for distinguishing altered gaseous environments. The increase that we noted in the survival rate of the "oxygen" mice in a gaseous mixture deficient in oxygen is partly related to lowered excitability of the nerve centers. It is known from the literature that resistance to hypoxia is higher in animals who are less excitable or who have been administered a dose of narcotic, neuroplegic, or sedative drugs (refs. 12, 13, 28).

Increase in resistance of animals to hypoxia and decrease in excitability of nerve centers is consistent with the slowing of the metabolic processes noted in our experiment: drop in the level of gas exchange, hypofunction of the thyroid gland, and lag in weight on the part of the "oxygen" mice. The aforemen- /528
tioned factors are known to help increase resistance to hypoxia (refs. 9, 21, 25, 29, 30).

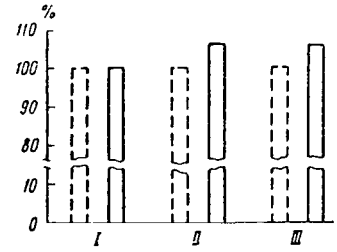

Figure 7. Relative weight of the animals' lungs following exposure to different partial pressures of oxygen (in percent of control); I, 0.6 atm (for 36 hours); II, 0.9 atm (36 hours); III, 2.5 atm (1.5 hours) .

The influence of oxygen on gas exchange and on the functioning of the thyroid gland, as disclosed in our experiments, also finds confirmation in the literature (refs. 14, 15, 40, 41, 53). These data bear evidence to modification of the metabolic processes and participation of the endocrine system brought on by exposure to a hyperoxic environment.

The enumerated functional changes also resulted in clear-cut changes in the blood morphology. The red and white blood cell series served as a fairly delicate test of the initial signs of the effect of oxygen.

The rather sharp changes in magnitude of the indices under study that occurred after the 6th day of the experiment as compared with the 10th are noteworthy. In the latter case, there is a tendency for the functional changes in the central nervous system and in the blood picture to level out. In our opinion, this fact may be considered an adaptative reaction by the organism to the action of high partial pressure of the oxygen towards the end of the 10-day experiment. On the other hand, the changes found in the blood may be due to the unfavorable action of the oxygen. Increase in the relative weight of the lungs and decrease in the number of reticulocytes is evidence of the initial manifestation of the specific toxic action of oxygen. This is confirmed by the onset of inflammation and edema of the lungs (refs. 5, 52) and by the suppression of hemopoiesis in bone marrow (refs. 27, 40) following exposure to high partial pressures of oxygen. This is also borne out by our experiments with higher oxygen pressures (0.9 and 2.5 atm). However, changes in the lungs and blood observed in our experiments are of a transitory character and cannot quite be considered pathological, especially as no inflammation was noted in the lungs. The relative increase in weight of the lungs in relation to body weight may be the result of hyperemia of the pulmonary tissue and distension of the alveolar capillaries.

The marked transitory changes in the level of gas exchange and in the blood picture following the abrupt replacement of a hyperoxic environment by air or by a hypoxic environment and in the converse manifestation of the same are noteworthy. The character of these changes may be regarded as a stress reaction to the unfavorable action of changes of the gaseous composition on the organism. /529
The resultant functional changes are temporary and they may be considered specific and nonspecific compensatory reactions aimed at increasing the resistance of the organism.

According to our experiments, the breathing of gaseous mixtures containing 63 percent oxygen for 10 days was relatively harmless. However, the end of this period saw changes which could be considered initial manifestations of the pathological action of oxygen. These changes affected primarily the lungs and blood.

Therefore, the choice of an artificial atmosphere containing 60 percent oxygen at atmospheric pressure for prolonged exposure of the organism would not be optimal from the physiological point of view.

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AMMONIA AS ONE OF THE COMPONENTS OF THE AIR MEDIUM IN CLOSED COMPARTMENTS

V. I. Mikhaylov

The literature shows that the presence of man in closed compartments is associated with the occurrence of ammonia in the air, the latter being released as a result of his vital activity (refs. 6, 8 and 10). The prolonged presence of man in closed, poorly ventilated compartments may increase the concentration of ammonia in the atmosphere to unsafe limits. In this connection, it becomes necessary to establish a maximum permissible concentration (MPC) for ammonia in the air of such compartments (refs. 7 and 12). It calls for the toxicological investigation of the substance in question. In the literature accessible to us, the toxic action of ammonia has not been sufficiently studied. In particular, the zone of its toxic action has not been established. Furthermore, contradictory data are found with respect to the accumulation of ammonia in the organism (refs. 2, 9, 11, and 14). /531

The study of these questions served as the object of our investigation.

Lethal concentrations of ammonia were determined in 170 male mice weighing 19-23 g each. Ammonia was supplied to the animals in concentrations of 2.38-6.3 mg/liter in an 8 liter glass chamber. The constancy of the ammonia concentrations in the chamber was maintained with the help of a device previously described (ref. 4). Quantitative determination was achieved, iodometrically. The experiments recorded the death of animals during administration of the ammonia and for a period of 15 days afterwards.

Animals subjected to lethal concentrations of ammonia showed irritation of the eyes and the upper respiratory tract accompanied by copious frothing at the mouth, motor disturbances and labored breathing. Moreover, the animals assumed a position which would give muscular assistance to the act of breathing (the mice rested on widely spaced front paws, gulping in air). This was followed by bodily tremor, disturbed motor coordination and development of clinical tonic spasms, terminating in death. /532

Autopsy showed pulmonary edema in a number of cases. The lungs were, as a rule, marble in color. The liver was enlarged, hyperemic and dark red in color. The stomach and intestines were distended and filled with a frothy substance.

The experiments showed that 4.51 mg/liter was the absolute lethal concentration of ammonia for male mice after a two-hour exposure, while 2.38 mg/liter

was the minimal acting. The tentative lethal concentration and its confidence limits, determined by processing the experimental materials by the method mentioned in reference 13, was 3.31 (2.86-3.84) mg/liter (P = 0.05).

Threshold concentrations of ammonia were determined with the aid of 100 white male mice weighing 20-24 g each. The ammonia was administered in the same way as in the determination of lethal concentrations. Evaluation of physiological and biochemical changes in the animals was based on the following integral indices: determination of body weight in action, resistance of the animals to a static muscular load and consumption of oxygen (ref. 3). Simultaneously, determination was made of blood catalase activity (ref. 1) and blood serum cholinesterase activity (ref. 5).

Changes in oxygen consumption of the animals following exposure to ammonia are shown in the table.

The table shows that when the experimental animals were exposed to ammonia in concentrations of 0.0072-0.0081 mg/liter, a statistically significant decrease in oxygen consumption took place as compared with the control experiment. However, in the background experiment, the difference in the magnitude of this index before and after the experiment was statistically insignificant. /533 Inasmuch as a distinct change in this index was observed at a concentration of 0.0081 mg/liter (from 3.46 ± 0.14 to 2.65 ± 0.36 mg/liter; P = 0.02), we took it as the threshold of acute action of ammonia on oxygen consumption.

An ammonia concentration of 0.0077 mg/liter produced initial modifications of blood catalase activity. Whereas the catalase index in the experimental animals increased from 1.11 ± 0.65 to 1.38 ± 0.10 (P < 0.001), it stayed within normal limits for the control animals.

Analysis of the data obtained in determining the resistance of the animals to a static muscular load showed that the action of ammonia in concentrations of 0.0072 and 0.0081 mg/liter produced an increase in this index from 58.46 ± 17.7 to 77.9 ± 12.1 and from 76.1 ± 11.54 to 82.13 ± sec, while the background experiment showed a marked decrease from 105.0 ± 14.87 to 59.6 ± 11.38 sec (P = 0.02)

INFLUENCE OF AMMONIA ON OXYGEN CONSUMPTION BY WHITE MICE.

Concentration of ammonia, mg/liter	No. of animals	Oxygen consumption, mg/liter of weight		Insignificance of difference P	
		Before experiment	After experiment	Before experiment	After experiment
Background 0.002	17	4.32 ± 0.26	3.81 ± 0.26	> 0.05	-
0.0072	10	4.22 ± 0.16	3.61 ± 0.25	0.05 > P > 0.02	> 0.05
0.0081	15	3.46 ± 0.14	2.65 ± 0.36	0.05	-

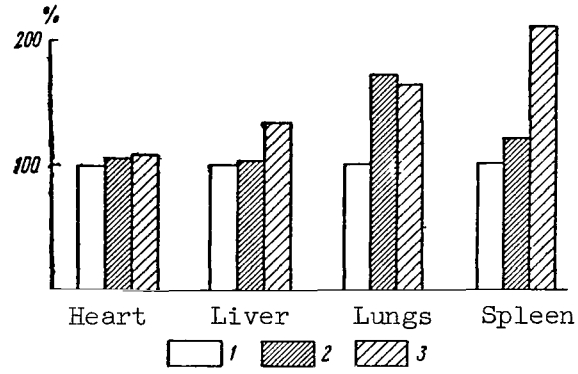
Blood serum cholinesterase activity did not change in these experiments.

The resultant data made it possible to calculate the size of the zone of toxic action of ammonia. It is equal to 4.50 mg/liter (4.51-0.01 mg/liter).

The true significance for the toxicology of a substance is the presence or absence of a cumulative effect. This matter was studied in experiments on white mice by the following method. The animals were divided into two groups of 15 each--experimental and control. The experimental animals were given ammonia in a concentration of 0.31 mg/liter 1/10 LC_{50} for 15 days, two hours per day. On the 16th day, the experimental and control animals were given ammonia in a concentration of LC_{50} (3.31 mg/liter). During the experiment record was kept of oxygen consumption, weight of the animals, deaths, weight coefficients of the heart, liver, lungs and spleen. Five animals died in the experimental groups starting the 8th day of administration; no animals died in the control group for the same period. Four animals each died in the experimental and control group following two-hour administration of ammonia in a concentration of LC_{50} (3.31 mg/liter).

On the second day 6 died in the experimental group and 3 in the control, and on the 6th day four mice died. Furthermore, the experimental animals were found to have an appreciably lower consumption of oxygen (to 58.5-76.2%) as compared with the initial level, especially during the first half of the administration period; this index remained within normal limits for the control mice. There was also a weight lag (8 percent) as compared with the control animals. Changes in organ weight coefficients are shown in the figure.

Determination of the organ weight coefficients in the experimental mice disclosed a statistically significant increase in the organ weight coefficients of the heart, lungs, liver and spleen.



Changes in organ weight coefficients of white mice during study of cumulative effect of ammonia. 1, intact animals; 2, control animals; 3, experimental animals.

Our experiments thus revealed in the experimental animals an increase in organ weight coefficients, decrease in oxygen consumption, weight lag and earlier deaths as compared with the control animals. It may therefore be concluded that ammonia has a cumulative effect. /534

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THE PROBLEM OF THE EXCITATORY STATE OF THE EMETIC CENTER IN MOTION SICKNESS

I. D. Pestov

A major role in the mastery of outer space is played by those /535
measures which will assure full working capability for cosmonauts. The orbital flight of the Soviet cosmonaut G. S. Titov gave us valuable information to the effect that weightlessness is conducive to the development of certain disorders, the symptoms of which are similar to those of motion sickness. Proper training regimes eliminated the onset of these symptoms in subsequent flights of 3-5 days' duration, thus assuring a high working capability for the cosmonauts.

At the present time, however, it would be premature to think that prevention of motion sickness in spaceflight has been completely achieved. A compelling need exists to study the mechanisms responsible for the onset of motion sickness, inasmuch as a thorough understanding of its pathogenesis would increase the chances of preventing it.

We know that the symptoms of motion sickness include a number of sensory, somatic and autonomic components. However, certain difficulties stand in the way of an objective exposition of the course of its development. Modifications in the functioning of the cardiovascular, respiratory and blood systems and in secretion, incretion and thermoregulation are not constant, but often vagarious (refs. 1, 5, 9 and 11). Biochemical methods and electroencephalography have the same defects (refs. 9 and 11).

A number of authors (refs. 9 and 10) have used the emetic reflex in their work for the study of developed forms of motion sickness. Because of its uniqueness as the culminating moment for the entire picture of the disease, vomiting has been subjected to exhausting study. It has been established (refs. 7 and 12) that vomiting in dogs, caused by rocking on swings, is pri- /536
marily related to the reception of impulses by the emetic center from the labyrinths by way of the vestibular part of the cerebellum (nodulus, uvula, pyramids) and the chemoreceptor zone of the emetic center located at the bottom of the fourth ventricle.

The role of other afferent influences in connection with the emetic center in motion sickness has not yet been completely elucidated. A certain role is probably played by impulses emanating from the organs of the peritoneal cavity traveling along the vagus and sympathetic nerves (ref. 12) and by optokinetic irritation. It is also known that the functional condition of the emetic center

may be considerably modified by the most diversified stimuli: visual, auditory (ref. 4), interoceptive (refs. 2 and 4) and musculocutaneous (ref. 3).

We assumed that the onset of vomiting in motion sickness is preceded by a change in the functional condition of the emetic center, namely its excitability. It was therefore necessary to develop a method for determining this excitability at different intervals following the impact of acceleration on the organism.

With information available on the dynamics of change in the excitability of the emetic center, it became possible to identify the developmental phase of motion sickness and to determine its latent forms, which could not be observed by other objective methods of investigation. Such an approach enabled us to determine whether changes in the functional condition of the emetic center are related solely to labyrinth afferentation or may involve other extralabyrinthine mechanisms capable of modifying the excitability of the emetic center during acceleration.

The following investigation was carried out with such a purpose in mind. The experiments used 8 mongrel dogs of both sexes weighing 6-14 kg. Five dogs had intact labyrinths and 3 had been delabyrinthed. This was done in one case by injecting a 1.5 percent solution of monoiodoacetic acid into the cavity of the middle ear and in two cases by surgical intervention (de Klein's method). Complete clinical incapacitation of the labyrinths was obtained in all three dogs.

Excitability of the emetic center while at rest and at different intervals during the experimental production of motion sickness was determined by the size of the minimal dose of apomorphine capable of inducing vomiting. A freshly prepared 0.05 percent solution of apomorphine hydrochloride was injected subcutaneously 1-3 hours after feeding. We conducted single apomorphine tests at intervals of 1-3 days, and frequently repeated tests at intervals of 20-30 min.

For the experimental production of motion sickness, we used mostly an irregularly rotating stand. During the course of a complete revolution it /537
would first pick up speed, then slow down and finally come to a complete stop. The whole cycle would then be repeated (table).

We also used the following stand: vertical oscillating (operating at 40-52 cycles/min with an amplitude of 35 cm); inclined rotation (angle of inclination of the platform with the animals ± 27° from the horizontal plane; operating at 40-50 cycles/min). The duration of the experiments on the stand was from 30-90 min.

We expressed the size of effective doses of apomorphine for this or that rotating or oscillating experiment in percentages of the threshold dose while at rest. In this way we obtained collative data characterizing the dynamics of change for the emetic center of different animals. Moreover, the number of vomiting attacks and their latent periods were also recorded. Records were made of electrocardiograms, respiratory movements and, in a number of cases, blood pressure of the carotid artery, exposed in a skin flap, and skin temperature.

SOME PHYSICAL CHARACTERISTICS OF ROTATIONAL SYSTEMS AT MINIMAL ANGULAR VELOCITY (0^{o}).

rpm	Duration of 1 turn, sec	Maximum angular velocity, ^{o}/sec	Mean angular acceler-ation, $^{o}/sec^{2}$	rpm	Duration of 1 turn, sec	Maximum angular velocity, ^{o}/sec	Mean angular acceler-ation, $^{o}/sec^{2}$
10	6	154	± 51	14	4.3	216	± 101
12	5	185	± 74	16	3.75	246	± 132

Altogether 140 experiments were carried out on the stands, during the course of which 400 apomorphine tests were made. Furthermore, apomorphine was administered 413 times during the intervals between experiments for the purpose of determining the size of the threshold dose.

The threshold dose of apomorphine during rest averaged 0.02 mg/kg. During the course of the experiment, only one dog began to require a sharp increase in the threshold dose of apomorphine, beginning with the 20th injection. By the 110th, it was 7 times the original amount. Despite the fact that the other animals received the same number of apomorphine injections (to 160), and in some cases even more, the threshold dose either remained the same or showed insignificant differences.

In all experiments we started with the threshold level which corresponded to the particular segment of time. We found that repeated administrations of apomorphine at 20-30 min intervals during the course of an experiment did /538 not affect the excitability of the emetic center.

In the intact dogs the rotating and oscillating procedures induced the development of certain external signs of motion sickness (licking, yawning, motor excitation or inhibition), but only episodic manifestation of vomiting.

The three delabyrinthed dogs failed to show these external signs of motion sickness. No patterns of change could be disclosed for the EKG, respiration, arterial pressure and skin temperature during the course of the experiments. The only exception occurred when the animals were nauseous or vomited. We shall describe these changes at the end of our report.

We found that, as rotational angular force and duration increased, the intact dogs required decreasing doses of apomorphine to produce vomiting. In this connection, however, each of the five intact dogs manifested individual differences based on its resistance to motion sickness. The graphs contain data characterizing the action of subliminal doses of apomorphine (expressed in percentages of the threshold dose) on three dogs sensitive to motion sickness at 10 rpm (fig. 1) and 12-16 rpm (fig. 2). The abscissa depicts the administration of apomorphine

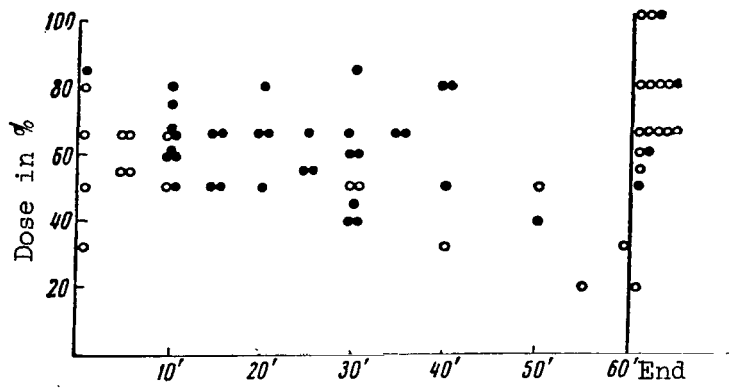

Figure 1. Action of subliminal doses of apomorphine on 3 intact dogs while rotating at 10 rpm.

in terms of time lapse following rotational actuation. The dark circles indicate doses that induced vomiting, the light circles those that failed to induce vomiting.

As shown in figure 1, the only effective dose at the beginning of /539 rotational actuation at 10 rpm measured 85 percent of the threshold dosage. After a lapse of 10-20 min, this dose dropped to 60 and 50 percent, and at the end of 30 min to 40 percent. Figure 2 illustrates the fact that, at 12-16 rpm, the excitability of the emetic center increases sooner and with greater force at 12-16 rpm than at 10. We note that subliminal doses of apomorphine were ineffective in most cases immediately after the rotations were stopped. However, after 12-16 rpm a number of cases showed a more frequent incidence of a residual increase in the excitability of the emetic center.

With regard to the excitability of the emetic center of the two dogs who were more resistant to motion sickness, the only effective doses at 10 rpm were those constituting 80 and sometimes 75 percent of the threshold dose. But at 12-14 rpm the size of the effective dose dropped to 66 and 60 and even 55 percent. Of the three delabyrinthed dogs, one showed high resistance to motion sickness, another average resistance (resistance in these two dogs being determined by external indications of motion sickness: unrest, salivation, licking and yawning), and the third extreme susceptibility. At 12 rpm the third dog vomited spontaneously in two experiments. At 10 rpm no spontaneous vomiting occurred, but after 20 min of rotation the effective dose dropped to 25 percent of the threshold dose.

The size of the threshold dose for these dogs, while at rest, was no different after the operation than before. However, in the rotating experiments at 10 rpm, not one of the subliminal doses, including the 80 percent one, /540 was sufficient to cause vomiting. Subsequent increase in rotational speed

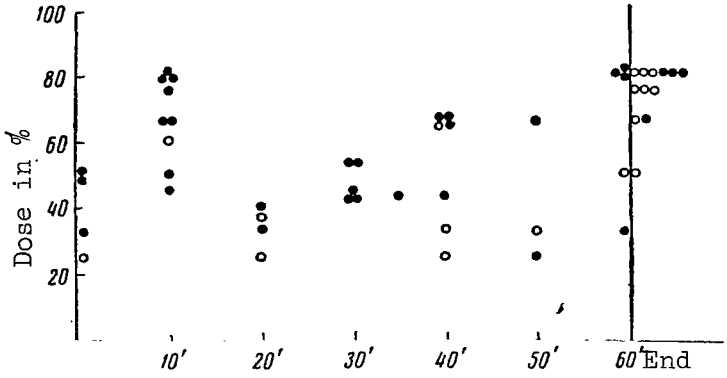

Figure 2. Action of subliminal doses of apomorphine on 3 intact dogs while rotating at 12-16 rpm.

enabled us to obtain a convincing picture of an increase in the excitability of the emetic center.

For the third dog an increase in the rate of rotation to 12 rpm was sufficient. This rate was not sufficient to produce spontaneous vomiting, but a dose equal to 80 percent of the threshold dose was effective after 10 rpm. This dog manifested in a number of experiments a kind of paradoxical reaction, to the effect that an 80 percent dose, while initially producing vomiting, lost its effectiveness after 30 min of rotation.

The second dog required an increase in the rate of rotation to 16 rpm to raise the excitability of the emetic center. At this rate, a 75 percent dose produced vomiting after 30 min of rotation, and a 50 percent dose after 40 min. Here we came across a paradoxical effect in isolated instances where the apomorphine test was positive at the beginning of the experiment and negative later. Even the injection of a 100 percent dose after a lapse of 20 min was ineffective in a number of experiments. We were able to produce vomiting in the first dog once at 16 rpm by injecting a 66 percent dose at the end of 50 min. Subsequent attempts to achieve this, even with an 80 percent dose, were unsuccessful. The action of a 100 percent dose of apomorphine on this dog, while under the influence of rotation, was not inhibited. Unfortunately, for technical reasons our apparatus was incapable of exceeding 16 rpm. For this reason we are unable at the present time to determine whether extralabyrinthine influences were lacking in this dog or whether our rotational speeds were insufficient to permit these influences to manifest themselves.

In order to elucidate whether any differences existed in the modifications of the functional condition of the emetic center as a result of the action on the organism of vertical rather than angular oscillation, we conducted a series of 29 experiments on stands with vertical oscillation. The experiments were carried out on 3 intact and one labyrinthectomized dog. We found out that this oscillation corresponded approximately in its effect on the functional condition of the emetic center to a rotation of 10 rpm. In two dogs sensitive to motion sickness, 50-60 percent of the apomorphine test dose was sufficient to

induce nausea after 10 min. Subliminal doses produced vomiting only with difficulty in the resistant dog. Subliminal doses were generally ineffective with respect to the labyrinthectomized dog, and even a 100 percent dose had a delayed effect (after 20 min).

It is interesting that under conditions of heightened excitability /541
of the emetic center during rotation or oscillation, both intact and labyrinthectomized dogs showed a significant decrease of more than 2 min in the mean values of latent periods of nausea attacks compared to attacks during rest. During rest, the relative number of repeated attacks did not change in the case of intact dogs, and was about halved for the labyrinthectomized dogs.

Excitation of the emetic center is accompanied by a number of characteristic changes on the part of the cardiovascular and respiratory systems. The qualitative aspect of these changes has been described by a number of authors (refs. 6 and 8). It consists of the fact that vomiting is accompanied by deeper breathing and acceleration of cardiac contractions. Furthermore, with the appearance of vomiting movements and expulsion of vomitus, breathing stops and bradycardia develops. During severe vomiting, the EKG shows signs of myocardial hypoxia. With cessation of vomiting, tachycardia is renewed and breathing becomes normal. After several minutes, the frequency of cardiac contractions returns to its original level.

Our experiments fully confirmed the particular character of these reactions.

Analysis of 136 cases of vomiting established that the pulse rate during the prevomiting period increased on the average to 260 beats per min. The mean quadratic deviation was ± 25.5/min, and the probability of attacks at pulse rates below 250 beats/min was only 0.15. In 103 cases where the administration of apomorphine did not induce vomiting but did show an increase in the pulse rate, the pulse rate reached 215 beats/min, with a mean quadratic deviation of ± 36/min. The probability of cases with a pulse rate of less than 250/min was 0.62.

We believe that in those special cases where vomiting must be induced by remote control, the mean indices for the pulse rate shown above may be of help in the diagnosis. If, against the background of a sudden attack of acute tachycardia, there is a brief, temporary period of bradycardia, such a symptom should be considered as pathognomonic of vomiting. Bradycardia in vomiting is probably related to the fact that at the moment of expulsion of vomitus, the glottis closes, and there is a sharp increase in intrathoracic pressure caused by forceful contractions of the thoracic and peritoneal muscles. The reflex mechanisms inducing in such cases retardation of cardiac contractions are sufficiently well known. It goes without saying that it would be more convincing to compare changes on the part of the cardiovascular system with changes in the functioning of respiratory and peritoneal muscles.

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PART IV. GENERAL PROBLEMS OF SPACE BIOLOGY AND METHODOLOGICAL ARTICLES

AN ELEMENTARY MODEL OF THE VESTIBULAR APPARATUS

O. G. Gazenko, N. A. Chekhonadskiy,
A. N. Razumeyev and B. B. Yegorov

An important part of the space orientation analyzer is the /543
vestibular apparatus which may be represented schematically as consisting of two sections--receptors, sensing certain type of effects, and the central section, connecting the receptors with different formations of the nervous system.

The receptor part consists of semicircular canals which react primarily to angular accelerations, and of the otolithic apparatus which senses the linear displacements of the head and body in space.

The sensitive element of the otolithic apparatus includes the accumulation of the epithelial filament cells in the region of the "auditory spot." The filaments of these cells appear to hold the moving otolithic membrane by their ends. Two otolithic formations are distinguished--the utricle and the saccule, which are inclined with respect to the horizontal and vertical plane. Although the reception mechanism in the semicircular canals is understood quite well, the function of the otolithic apparatus is rather controversial at this time. Several hypotheses exist, but they require further experimental verification.

The most widely held concept assumes that the reception in the otolithic apparatus is achieved by the displacement of the otolithic membrane with respect to the receptor cells.

At the present time the mechanism for the encoding of mechanical energy during the displacement of the membrane into an electric process, which /544
occurs in the receptor cell, is completely obscure.

The purpose of the present work is to develop a simple model of the vestibular apparatus to clarify certain properties of this organ when a variable gravitational field acts on the organism. This, of course, is of definite interest in space medicine.

We shall present some of the results obtained in our work, which was directed towards developing the basic requirements to be satisfied by such a model. We primarily consider the function of the otolithic part of the vestibular apparatus.

1. Receptor Characteristics of the Otolithic Part of the Vestibular Apparatus

In developing a model of the vestibular apparatus we formulated a series of working hypotheses which explain the structure and function of some elements of the otolithic part.

Our work was based on experimental data. In the investigations the action potentials on the receptors and on the neurons of animal vestibular apparatus were measured when the animal organism was subjected to mechanical forces (ref. 1).

During the recording of action potentials of individual efferent fibers in the macula utriculi it was established that with the slightest motion of the animal's head in various directions there is a substantial variation in the frequency of impulses, and that impulsation during vertical displacement is proportional to the amplitude of oscillations.

One of the hypotheses assumed by us is the proposition on the "structure" and operating principle of the receptors in the otolithic part of the vestibular apparatus.

From the analysis of these experimental data we can state that all receptors of the otolithic part of the vestibular apparatus (both of the utricle and saccule) consist of generator sensors, which transform the angular displacement of the head (of the animal or of the human being) from the vertical, into electric impulses whose frequency is proportional to this angular displacement.

In regard to matters which directly concern the "structure" of /545
these receptors we have assumed a hypothesis which essentially states that the receptor reacts to the inclination of the head with respect to the vertical because there is a change in the magnitude of the gravity force components acting on the otoliths. These force components are directed along and across the filaments which are in some manner coupled to the otoliths (fig. 1). The transducers which convert these forces into the frequency of impulses are the sensitive cells.

With such a "structure" the receptor will react both to the deviation of the head and to the value of acceleration taking place when the animal moves, since in this case there will also be a change in the forces acting on the otolith. This is precisely what was determined experimentally when our work was carried out with animals. We found that the frequency of impulses increased proportionately to the G-force.

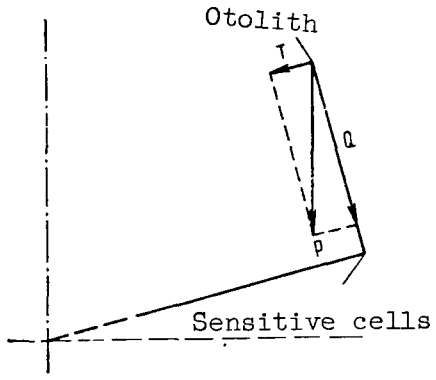

Figure 1. Schematic representation of receptor. P, weight of otolith; Q, weight component directed along filament; T, weight component transverse to filament.

2. Reaction of a System Consisting of a Receptor and a Neuron to a G-Force Which is a Step Function of Time

Let us consider the characteristics of a network consisting of a receptor and a series of neurons connected in series. In this case, in our investigations the action potential was measured for neurons of the second order. In the future we shall refer to this network as the receptor-neurons system.

To determine the dynamic properties of this system we analyzed the variation in the rhythmic activity of 100 neurons associated with the giant-cell nucleus of the reticular formation, when the organism of an animal (of a cat) was subjected to a constant acceleration of 5 G.

The initial reaction was usually manifested as an increase in the frequency of impulses (the activation phase); this was followed by a phase in which the impulses were grouped into "packets" separated by periods of "silence," then the pulse activity of the neuron ceased completely and the "silence" phase took place. The last phase was retained during the entire period of time while the animal was subjected to this acceleration.

After the G-force was removed, the opposite picture was observed. Samples of potential readings showing the action of neurons in these cases are shown in figure 2.

The average data obtained by measuring the activity of 10 neurons with loads of 5 G are shown in figure 3.

The experimental relationship showing the variation in the rhythmicity of the neurons, when the animal organism was subjected to a constant G-force, may be represented as an additive result of two processes taking place simultaneously in neurons. The first process produces an increase in the number of impulses, the second produces a decrease, but its development is delayed with respect to the first process.

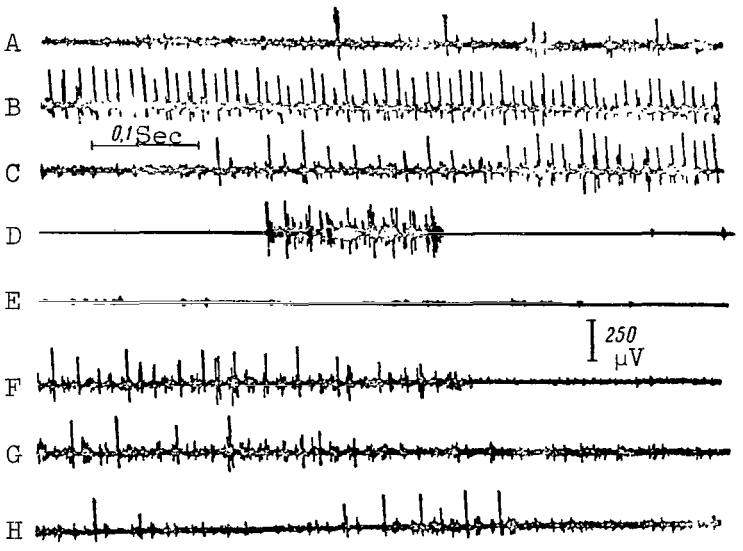

Figure 2. Variation in rhythmicity of neuron associated with giant-cell nucleus of reticular formation when loads of 5 G are acting. A, initial rhythmical activity; B, activity after 35 sec of rotation (15 sec of area); C, 75 sec of rotation; D, 90 sec of rotation; E, 120 - 150 sec of rotation; F, after centrifuge stopped; G, 20 - 50 sec after centrifuge stopped; H, 150 sec after centrifuge stopped.

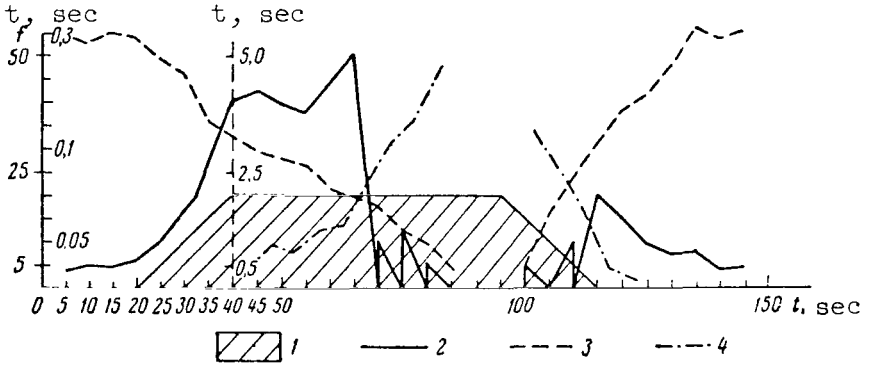

Figure 3. Average data on variation in rhythmic activity of neurons associated with giant-cell nucleus of reticular formation during action of transverse loads of magnitude 5 G. 1, G-forces; 2, frequency of impulses; 3, intervals between impulses; 4, intervals between groups of impulses.

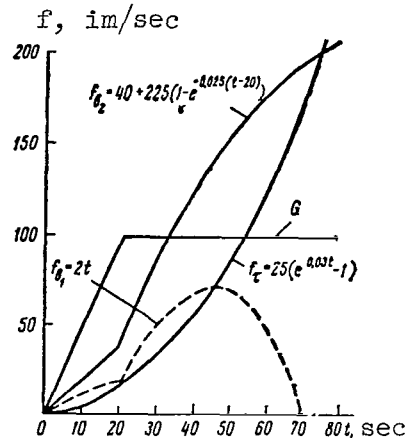

Figure 4. Graphic representation of processes developed in neuron under action of G-forces.

Figure 4 shows curves which illustrate the relationships obtained by us from the mathematical model used to simulate the processes which take place in neurons, as described above. We see that the overall curve showing the neuron impulse frequency, when a constant force of 5 G acts on the animal organism, is sufficiently close to the experimental curve shown in figure 3. /547

It is interesting to note that our model of the processes taking place in the neuron under the action of a constant G-force may be used to obtain a relationship for the restoration of its background rhythmicity after the action of the G-force has ceased.

The graph in figure 5 shows this relationship; it was obtained by adding two curves describing the processes in the neuron based on the properties of the model.

We should point out that a similar approach to the investigation of stimulation and inhibition processes taking place in the central nervous system is used in the work of G. V. Savinov and his co-authors (ref. 2).

3. Reaction of the Receptor-Neuron System to G-Forces which Vary Periodically with Time

The question concerning the dynamic properties of "canals" in the otolithic part of the vestibular apparatus is of great interest. Based on available experimental material we can propose that the electric model of the receptor-neuron "canal" as a first approximation may consist of a network with resistance (R), inductance (L) and capacity (C) connected in series. The G-force acting on the organism of the test animal will be equivalent to the voltage connected to the input of the network, while the current in the network will represent the electrical activity of the receptor-neuron system, i.e., the frequency of recorded impulses. /548

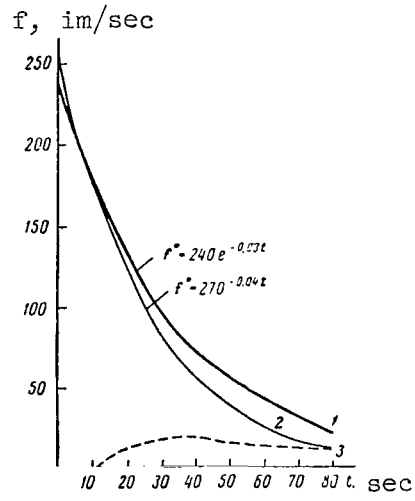

Figure 5. Graphic representation of processes in individual neurons of reticular formation after G-forces have ceased to act. 1, frequency of impulses during process of neuron excitation; 2, frequency of impulses during inhibition of neuron; 3, overall curve for neuron impulse frequency.

It is well known that the current in such a network during the transient process varies, depending on the parameters of the network and on the amplitude, frequency and phase of the sinusoidal voltage fed at the input to the network. Depending on the relationship between these quantities, the nature of current variations in the network may be quite diverse. We shall consider a single case when the relation between the parameters of the receptor- /549 neuron canal is such that they form an aperiodic network, the G-forces follow a sine law, and the pulse frequency produced by the G-force when it first starts to act is equal to zero.

The graphs in figure 6 show the components of the impulse frequency which arise in our model and also the total curve of this frequency. These data indicate that in our case the impulse frequency at the output of the receptor-neuron system must vary periodically, together with the variation in the frequency of the G-force. Also, at the beginning of its action we observe an increase in total amplitudes, compared with those in the steady state process.

The experimental investigations which we carried out confirm the possibility of this phenomenon. The relationships obtained for two receptor-neuron networks are shown in figure 7. By considering the properties of the model which simulates the receptor-neuron system in the dynamic state, we can explain the principle which is the basis of the phenomenon responsible for the presence of receptor-neuron system canals in the otolithic part of the vestibular apparatus. These react with their increased impulse activity when the animal moves in a definite direction (up or down) and decrease the impulse activity when motion takes place in the opposite direction. The

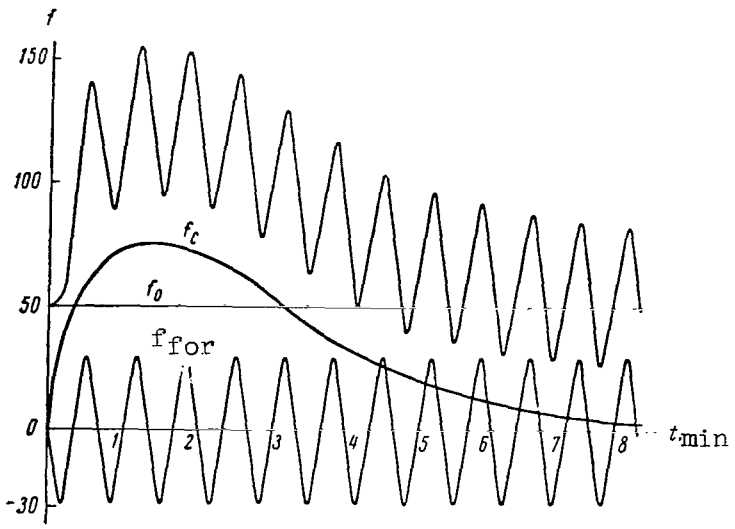

Figure 6. Graph showing variation in impulse frequency in crude model of biological system when variable G-forces are acting. f_0, constant component; f_{for}, forced component; f_c, free component.

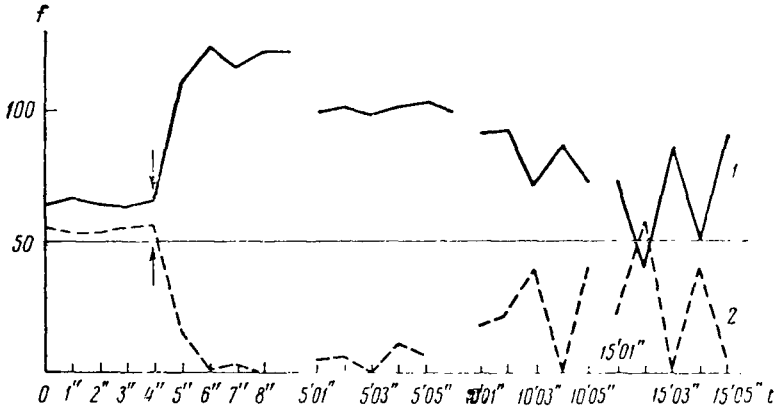

Figure 7. Variation in rhythmical activity of neurons during prolonged stimulation of otolithic apparatus. 1, network No. 1; 2, network No. 2. Along vertical axis, number of impulses per second; along horizontal axis, action time of periodic force.

reason for this process is also the existence of relationships between /550
the parameters of the receptor-neuron systems and the parameters of the mechanical force which acts on the organism of the test animal. When these relationships are of a certain type, the output quantity of the receptor-neuron system (impulse frequency) may be in phase with the input quantity of the system, i.e., the impulse frequency will increase with the G-force (movement up). However, for other receptor-neuron systems with different parameters the system may be out of phase with the input quantity during the same frequency of mechanical oscillations. In this case, if the G-force is increased (motion up), the impulse frequency of the receptor-neuron system will decrease. If the motion is "down," the impulse frequency will increase. Thus the receptor-neuron system with these properties will react only to "down" motion, unlike the previous system.

However, this phenomenon will take place only at a specific frequency of the mechanical forces acting on the animal organism. If the frequency of these oscillations is changed, the phase relationships in the receptor-neuron system may be different.

4. The Purpose and Some Characteristics of the "Summation Device"

The basic feature of the model for the otolithic part of the vestibular apparatus is the presence of a certain "summation device" (fig. 8), which compares in pairs the signals arriving from the left and right utricle and from the saccule. One of the functions of the summation device may be explained by considering the diagram shown in figure 9.

During the vertical position of the animal's or bird's head, the signals from the left and right utricles are equal; therefore, the output value of the summation device is equal to zero. When the head is inclined to the left or right by an angle α with respect to the horizon, the output quantity of the summation device is determined by the following relationships:

(a) for the "canal" which reacts to the force directed along the filament

$$\Delta f_Q = 2k_Q P \sin\alpha \sin\beta, \tag{1}$$

(b) for the "canal" which reacts to the force directed across the filament

$$\Delta f_T = 2K_T P \sin\alpha \cos\beta, \tag{2}$$

where P is the weight of the otolith; /551

k_Q is the sensitivity factor of the receptor along and across the filament;

α is the inclination angle of the head (in the frontal plane) with respect to the horizon;

β is the inclination angle of the utricle plane with respect to the horizon.

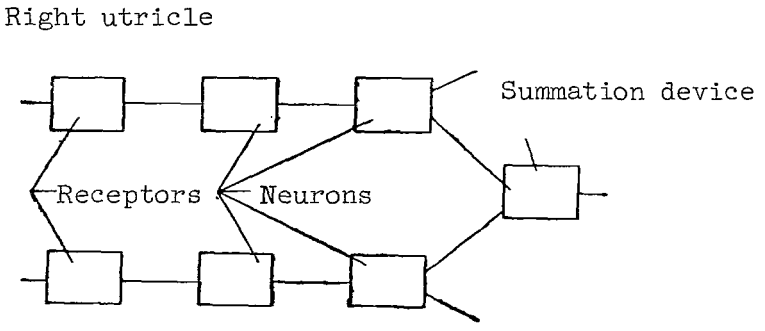

Figure 8. Diagram of summator.

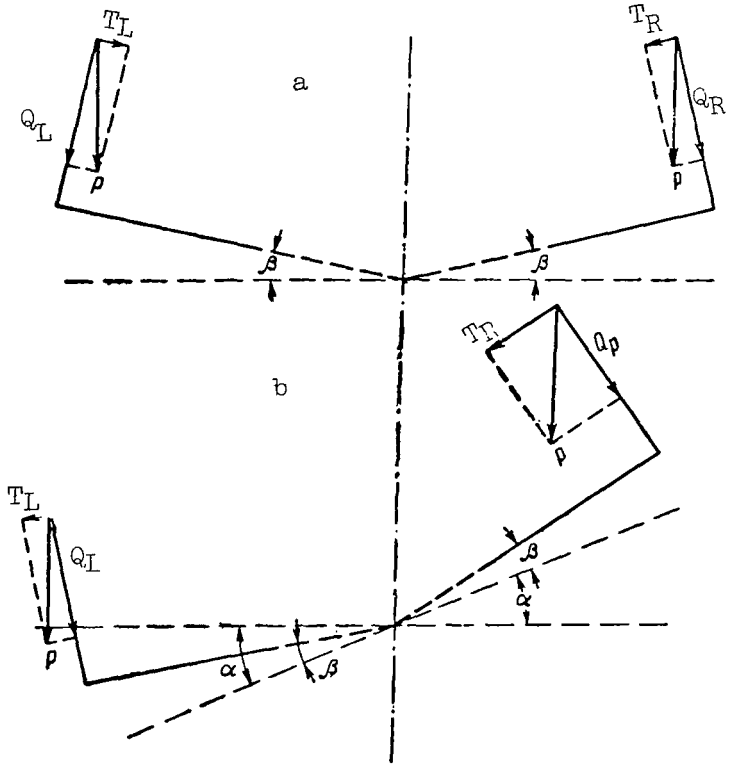

Figure 9. Function of the summator under static conditions. a, vertical position of head; b, inclination of head to right by angle α.

The relationships which have been presented explain some of the special features of the device and the function of the otolithic apparatus, specifically:

(1) Depending on the direction of the animal's head inclination with respect to the vertical, there is a change in the signs of the added quantities in the canal, which reacts to the force directed across the filament. This produces a substantial variation in the output quantity of the summator which apparently is used in the central nervous system to determine the direction and the inclination angle of the head with respect to the usual vertical position;

(2) During the inclinations of the head, the presence of the angle β produces the differences between the signals which are fed to the input of the summator from the left and right utricles along the canal which reacts to the force directed along the filament. When this angle is absent, the output quantity of the summator along this canal is always equal to zero. In this case, the capacity of the receptors to generate electric impulses proportional to the force component along the filament is not utilized.

Let us consider the function performed by the summator of the vestibular apparatus during the flight of a bird in a circle. In this case (fig. 10), different forces will act on the right and left otolithic devices. If a left bank is performed, the centrifugal force N_n acting on the right otolith may

be represented by the relationship

$$N_n = (r + 0.5l\cos\alpha)\,\omega^2, \tag{3}$$

where r is the turn radius;

l is the distance between the right and left otolith;
ω is the angular velocity of the bird during banking.

The left otolith will be subjected to the force /552

$$N_L = (r - 0.5l\cos\alpha)\,\omega^2. \tag{4}$$

Since the pulse frequency in the receptors of the vestibulary apparatus is proportional to the force acting on the receptor, the output quantity of the summator comparing both frequencies may be represented in the following manner

$$\Delta f = kl\omega^2\cos\alpha. \tag{5}$$

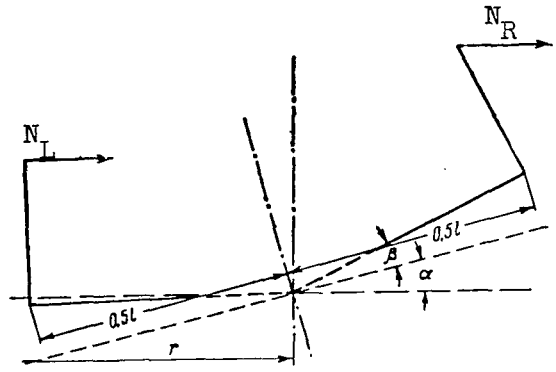

Figure 10. Function of the summator during motion. N_L, N_R, centrifugal forces acting on left and right otolith; r, banking radius; l, distance between left and right otolith.

The value obtained by us for the output quantity of the summator may explain to some degree the functions and operating principle of mechanisms which control the flight organs of a bird. In analyzing this expression we see that if the control system is adjusted for some signal Δf_{opt}, corresponding to a bank without slipping, then, if this maneuver is to be performed with a /553 high angular velocity and if the same value Δf_{opt} of the output quantity is to be maintained, it will be necessary to increase the angle of inclination with respect to the horizon. This relationship actually exists when birds or flying machines constructed by man perform a banked turn.

It is interesting to note that the presence of the summation device, which operates in this manner, explains very well the nature of motion by animals and birds whose right and left otolithic apparatus has been removed.

Indeed, in the case of the left bank considered above, the frequency of impulses in the filaments of the vestibular nerve, fed to the input of the summator, exceeded the frequency of impulses in the left vestibular nerve, which caused the flight organs to go into a state corresponding to the left bank. If the left otolith apparatus is removed, the sign of the output quantity of the summator is conserved, i.e., the flight control organs during horizontal flight are immediately oriented to produce a left bank.

Since the left otolithic apparatus is removed, the input of the summator will not be fed with the "negative value" of the impulse frequency produced by this device, and the output quantity of the summator achieves a substantial value compared with the norm. If during the banking maneuver the bird's flight organ control system is, indeed, tuned to some optimum value of the summator output quantity, then in this case, as follows from expression (5), the bird with a removed left otolith would tend to increase its angle α up to the maximum value of 90°.

It has been shown in the works of R. Magnus (ref. 3) and others that /554 these phenomena do occur, apparently confirming our propositions.

The material presented shows that the principles for the model of the otolithic part of the vestibular apparatus explain certain general properties of this important organ. A further development of this question, we hope, will make it possible to construct an electric model necessary to conduct rather extensive investigations of the functions performed by the vestibular apparatus.

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SEMICONDUCTOR COOLER FOR SMALL ANIMALS

Yu. N. Logunov and Yu. S. Alyukhin[1]

Existing methods of cooling the body for artificial hypothermia do /555 not permit rapid, simple changeover from cooling to heating, or smooth control of the temperature gradient. Cooling and heating devices are complicated and cumbersome.

The thermoelectric method of cooling and heating makes it possible to create a small-sized cooler with even temperature control. Simple reversal of the direction of current flow suffices to change over from cooling to heating and vice versa.

The theory and practice of thermoelectric cooling (TEC) were first developed at the Institute of Semiconductors, Academy of Sciences, USSR, whose staff has created a large number of TEC devices for use in various branches of science and technology. Among those intended for physiology and medicine, three merit mention:

(a) thermoelectrically cooled microtome platforms;

(b) the "thermod," a device for temperature stimulation of the skin; and

(c) a microcooler for treating skin diseases.

These semiconductor devices exploit Peltier's effect, which consists of the fact that if a direct current is passed through a thermoelectric circuit consisting of different kinds of conductors, an amount of heat Q_n (supplemental to Joule heat) is liberated or absorbed at the juncture points, depending on the direction of the current.

The quantity of heat absorbed at the conductor interfaces is proportional to the current strength and the duration of flow: $Q = \Pi It$. The coefficient of proportionability Π, called Peltier's coefficient, depends on the physi- /556 cochemical properties of the circuit's conductor materials and is numerically equal to the thermal energy released or absorbed at the interface during passage of a unit quantity of electricity.

[1]Design and manufacture of the device was accomplished by Yu. N. Logunov. Yu. S. Alyukhin drew up the experiments for cooling the animals.

Peltier's effect is especially pronounced in circuits consisting of electron and hole semiconductors. The Institute of Semiconductors has developed materials with good thermoelectric properties. Some of the best semiconductor materials at the present time are intermetallic alloys containing bismuth, tellurium, antimony and selenium.

TEC installations consist of three basic parts: a thermoelectric battery, a working chamber or surface, and a system for removing heat from the thermobattery.

The first of these consists of a number of thermal elements connected in parallel or in series. The thermal elements with electron conductivity (negative branch) are connected in series by bus bars with the elements having hole conductivity (positive branch), alternating positive with negative. There is a separate system of bus bars on each side of the thermobattery. When a current flows through the two sides of the thermobattery, that set of bus bars which corresponds to the system of "cold" junctions in the thermal elements is cooled, while the other set, corresponding to the "hot" junctions, is heated. The hot bus bars are the part of the thermal elements which rests against the system for heat removal from the battery. The heat from the hot junctions of the battery can either be dissipated by an air radiator or removed by a cooling sleeve in which running water circulates.

The working chamber of the device is made from copper or aluminum possessing good heat conductivity.

The semiconductor cooler for immersion of small animals for cooling to a state of hypothermia, described in the present article, is the first experimental model of a device which permits regulation of the rate at which the body is cooled. The device consists of three basic parts: a thermobattery, a small bath for the animals and a chamber (fig. 1).

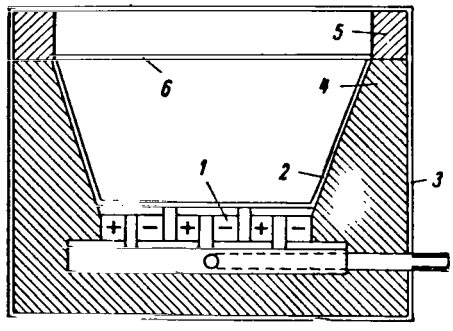

Figure 1. Schematic representation of semiconductor cooler for small animals: 1, battery composed of thermal elements joined in series with electron (-) and hole (+) semiconductors; 2, bath for animal; 3, vinyl plastic chamber; 4, heat-insulating layer of foam plastic; 5, upper cover; 6, plexiglass window.

The "hot" bus bars of the thermobattery stand on a copper plate 12 mm thick through which channels for water flow have been drilled. The water is supplied through rubber tubes to the inlet tap and passes out through another tap with drain tube attached. In this manner heat is removed from the battery junctions. The "cold" bus bars make up the base on which the copper bath is placed. Electroinsulation in the design is provided by using epoxy glue and fluoroplastic.

The entire TEC installation is mounted in a chamber made from vinyl plastic (3), which is protected by a heat insulation layer, made from PS-1 plastic foam (4), for reducing heat flow from the ambient medium. /557

The lid (5), with a plexiglass window (6), permits observation of the test animal.

The semiconductor cooler must be fed by high amperage, low voltage dc. During tests on the device, a VSP-33 selenium rectifier was used as a power source. By changing the current strength, the cooling rate for the animal placed in the bath can be smoothly regulated.

The principal technical data of the semiconductor cooler for small animals are: working current, 25 A; voltage drop through the thermobattery, 0.4 V; required power, 24 W.

The dimensions of the chamber are: height, 160 mm; width, 120 mm; length, 210 mm. The weight of the device is 2 kg.

For experimental purposes white rats were used, the majority weighing from 180-200 g. Four sets of experiments were made. In three the rats were anesthetized by subcutaneous injection of a 5 percent solution of hexenal. Body temperature was measured by a mercury thermometer inserted into the rectum to a depth of 5 cm or by a thermistor inserted to a depth of 3 cm. The cooling rates for typical tests of each series are illustrated in figure 2.

In the first series (6 tests), we determined the cooling rate for a /558 rat anesthetized at room temperature (17-19°C). For rat No. 6, which received a hexenal dose of 0.9 mg per g body weight, the average cooling rate (typical for this series) was 0.15°C per min until attainment of a rectal temperature of 27°C.

In the next three series hypothermia was produced with the cooler working at full power.

In the second series (6 tests), hexenal was introduced in doses of 0.2 to 0.7 mg/g body weight, and the rat was placed in the cooler (as a rule immediately after loss of the postural reflex). With such doses, depression of thermoregulatory reactions was inadequate: violent shivering continued right down to 27°C, and after the rat was placed in the cooler the postural reflexes were /559 restored. In a typical test of this series (curve 2), the cooling rate averaged 0.17°C per min, reckoning from the moment of placement in the cooler to attainment of a rectal temperature of 27°C; in other words, there was little change from the first trial.

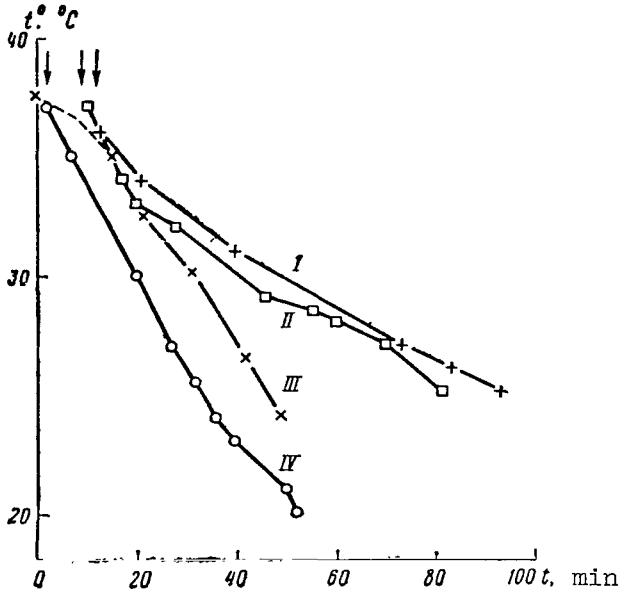

Figure 2. Body-cooling rate: I, rats anesthetized at room temperature (19°C) after hexenal injection 0.9 mg/g; II, rats anesthetized in cooler after 0.7-mg/g injection; III, rats anesthetized in cooler after 0.9-mg/g injection; IV, body of rat placed in cooler immediately after being sacrificed. Abscissa indicates time after beginning of injection or sacrificing; ordinate, rectal temperature. Initial part of third curve estimated from existing portion.

In the third series (9 tests), the rats were placed in the cooler after a hexenal injection of 0.9 mg/g body weight. Adynamia in this case was more complete and there was less shivering. In a typical case (curve 3), the cooling rate averaged 0.31°C per min from placement in the cooler to attainment of 27°C.

Since the principal obstacle to increasing the cooling rate is persistence of thermoregulatory reactions in anesthetized animals, we conducted two experiments in cooling the body of a rat immediately after its death. In one experiment (curve 4), we averaged 0.39°C per min down to 27°C; in the other, 0.48°C per min down to 24°C.

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ELECTRICAL METHOD OF RECORDING TONGUE MOVEMENTS DURING ARTICULATION OF CONSONANTS

Yu. I. Kuz'min

It is obvious that the possible logic for the automatic perception /560
of speech cannot be clarified without a detailed investigation of the time structure of speech signals. The realization of such an investigation at the acoustic level is associated with tremendous difficulties. At the same time, data which are analogous in principle could be obtained by methods of recording the articulation motions. It is clear that in order to investigate the time characteristics of the speech process such a recording must be continuous and must take place under conditions close to the natural conditions of speech formation. However, until now there has been no simple and reliable method of recording articulation motions during the process of coherent speech. Efforts to adapt electromyography to this purpose have yielded no positive results to date (refs. 1, 5 and 8). The application of X-ray motion pictures gives fairly good results for the solution of a series of experimental problems (in particular, some data on the time structure of vowels were obtained exactly by this method); however, the scope of application of X-ray movies is limited by the extreme complexity of this method, by the difficulty of processing the data and the difficulty of correlating articulation data with some image of the acoustic signal.

It is admitted that one of the most complicated problems is the development of a methodology for recording the motion of the tongue. At the same time the role played by this organ in generating most of the speech sounds is paramount. The positions of the tongue during articulation of vowels have been investigated by phoneticians primarily by the method of palatography.[1]

The palatogram--the image of the tongue contact zone with the hard palate-- /561
quite accurately determines the place and to some extent the method of articulation of most consonants. A method to obtain this type of data continuously during speech generation would be a most effective means for investigating the speech process. The first efforts to make palatography continuous were made by Stetson (ref. 13). Stetson used artificial palate plates containing rubber bulbs connected with tubes to a Marriott bottle. One such bulb was placed at the front edge of the palate, while the second one was placed at the rear edge. During articulation of prepalatal and postpalatal contact, the bulbs were compressed by the tongue, which produced the deviation of the indicator installed in the Marriott bottle. In view of its special features, the pneumatic recording

[1]In classical palatography a plastic plate which corresponds exactly to the configuration of the palate is powdered with talcum and inserted into the mouth of the subject. After each required sound is pronounced, the plate is removed and the zone where the tongue made contact with the palate is copied on paper.

method of Stetson determines the duration of existing contacts, with limited accuracy, during the articulation of explosive consonants, but does not determine the configuration of the zone where the tongue makes contact with the palate.

We developed a sensor to record the existence of contact between the tongue and the palate at several places simultaneously under conditions of continuous recording (ref. 6). The essence of this methodology, which has been called dynamic palatography, consists of the following. Registration is accomplished by means of a multichannel electric sensor consisting of a thin plastic plate firmly attached to the palate of the subject and containing electrodes on its surface. The plates are custom built for each test subject in the same way as palate plates for stomatological application of prosthesis. The electrodes situated on one-half of the sensor are fed with an ac voltage, while the electrodes situated on the other half of the sensor develop a signal at the time the tongue touches the respective zone of the plate. The sensor is connected to a measurement device, developed by V. A. Kozhevnikov and V. S. Shuplyakov (ref. 4), which amplifies, detects and smoothens the generated high frequency signal. After this the signals are recorded by means of a multichannel pen recorder and can also be measured by a series of electronic devices. The diagram of one of the channels for recording the signals from the sensor is shown in figure 1.

Such sensors were fabricated and tested in experiments with six subjects. The typical dynamic palatograms of words are shown in figures 2a and b. Each of the recorded parameters corresponds to a definite point on the hard palate. The presence of a signal corresponds to the presence of a contact between the tongue and the palate at a given point, while the absence of the signal shows contact is absent. Thus, the configuration of the contact zone can be reproduced from the available combination of signals at the output of the sensor at any given instant of time.

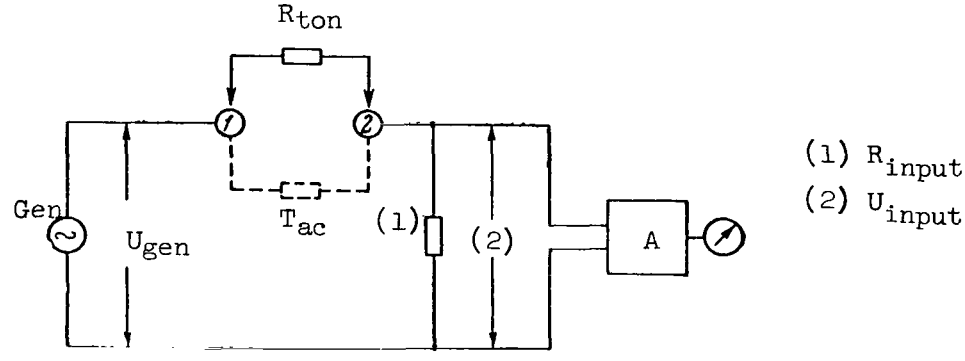

Figure 1. Diagram of one channel in artificial plate sensor; 1, electrode supplied with voltage; 2, electrode from which voltage is read; Gen, audio frequency generator; R_{ton}, resistance between electrodes when tongue touches palate; T_{ac}, resistance between electrodes when there is no contact between tongue and palate; A, amplifier with pen recorder.

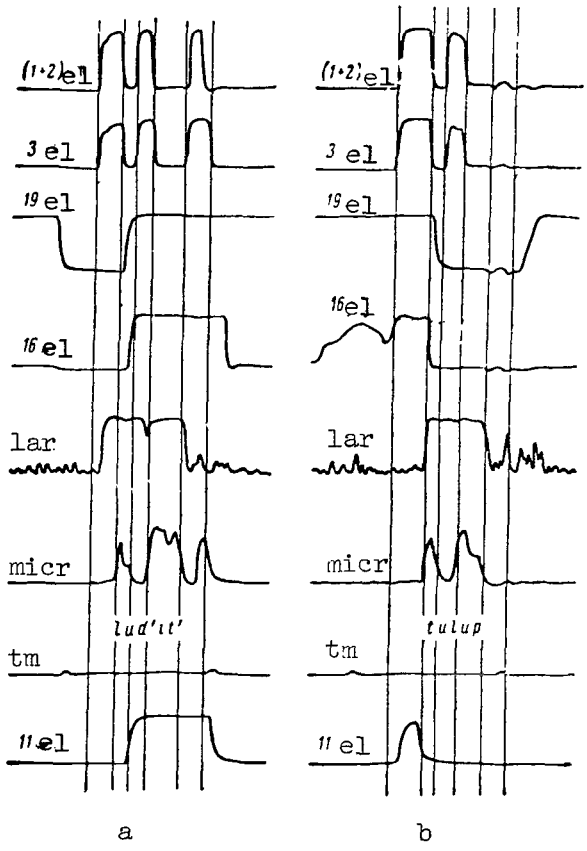

Figure 2. Dynamic palatograms of the words "lud'it'" (a) and "tulup" (b). Recorded parameters are: (1 + 2) el, 3 el, 19 el, 16 el, 11 el, electrodes of artificial palate sensor; lar, envelope of signal from throat microphone; micr, envelope of signal from microphone; tm, time marker.

This method continuously records the position of the tongue during /562
the articulation of most consonants, also of high pitch vowels in the process of coherent speech. This makes the time structure of consonants and the special features associated with their articulation in different phonetic positions accessible for objective investigation. In addition, the recorded signals can be used as reference points in the investigation of the more general characteristics of the speech formation process. Thus, the method proved to be useful in carrying out a series of psychophysical experiments associated with the study of speech formation mechanisms and the reception of speech by man (refs. 9 and 10).

Since in the course of such investigations it becomes necessary to process a large amount of data, the manual processing of information becomes rather difficult, and the measurement of the time characteristics of the process cannot always be accomplished visually with sufficient accuracy. In view of this, the present work was undertaken with the purpose of investigating the possibilities for the automatic processing of data obtained by means of a dynamic palatography

and to determine such recording methods which would be most suitable from this point of view. In the simplest case the automatic processing is reduced to the detection of the beginning or of the end of signals in different channels of the sensor and the measurement of time intervals between them. In the more complex case the signals to be measured must correspond to complex phonetic phenomena and must be formulated by a device with a built-in logic.

Obviously the possibility of applying automatic processing depends /563 on the reliability with which the useful signals are detected on the background of possible noise, the accuracy of determining the beginning and the end of signals and the degree of correspondence between the recorded signal and the phonetic phenomena being investigated.

To determine whether the useful signal is always detected during the automatic processing we established the variation limits of the signals and the level of disturbance. In considering the possible interference during /564 the recording process we must include the presence of residual conductivity when the tongue is not in contact with the palate. The latter is produced to a large extent by moistening of the sensor surface. When dynamic palatograms are processed visually, in the overwhelming majority of cases it is not difficult to determine which particular channel carries a signal. The magnitude of residual conductivity is relatively small and remains quite stable during the recording session. However, we should expect to have some variation in the value of residual conductivity in the course of the recording. In addition, the amplitude of the useful signal may also vary for a series of reasons: due to oxidization of the electrodes, due to change in the moisture content of the mouth and due to different contact pressure between tongue and palate. It is clear that if there is a large variation in the signal-to-noise ratio, the automatic detection of signals could not be carried out with reliability. We believe that the conditions for detecting the signals can be increased by properly selecting the magnitude of the voltage fed to the sensor. We could vary this quantity within definite limits, but since the nature of the relationship between the signal-to-noise ratio and the magnitude of the voltage was not clear, the optimum value of the voltage could be established only by experimental means.

The accurate determination of the beginning and end of signals is directly associated with these factors. Since the signals used in automatic processing must be obtained by smoothing out the high frequency carrier, the frequency of this carrier turns out to be an additional factor which determines the possible accuracy of measurements. The higher the carrier frequency, the lower is the time constant in the smoothing operation and the more accurate is the determination of time when the tongue contacts the palate. However, the use of high frequencies is limited by the possibility of capacitive coupling between conductors and the consequent generation of additional interference. It is desirable to select the carrier frequency which will make the time constant of the smoothing operation a minimum without producing any additional interference.

To determine the range of residual conductivity, the values of voltage were measured on two electrodes of the sensor (No. 1 and No. 19) with and without contact over a substantial period of recording time. The same test subject

was used during these measurements, because we were interested in processes associated with the construction of the sensor and the recording conditions. These obviously do not depend on the speaker. Measurements were carried out with a vacuum tube voltmeter connected to the output of the sensor. Measurements were taken every four minutes for a period of one hour.

The data obtained are shown in figure 3. As we can see, the conductivity between the various electrodes varies considerably, but is usually one /565
order of magnitude greater than the residual conductivity. It is interesting to note that when there is no contact between the tongue and the palate, the conductivity depends very little on the distance between the test electrode and the electrodes supplied with the voltage. This is significant, because it makes it possible to use identical circuits during the automatic detection of signals in different channels.

These quantities were obtained with an input voltage of 250 mV. In order to determine more accurately the magnitude of the voltage which provides for a more favorable signal-to-noise ratio, the following measurements were carried out. With the different voltages supplied to the current sensor, the signal level and the noise level were measured in the three different channels of the sensor, while two speakers pronounced the same syllable (ta). The applied voltage was varied in steps from 50 mV to 1 V. The results of the measurements showed that the noise level in the entire investigated range of input voltages remains relatively low. Since during this time the intensity of the signals increases proportionately to the input voltage (fig. 4), the conditions for detecting the signal, as /566
the voltage to the sensors is increased, become substantially better. Since

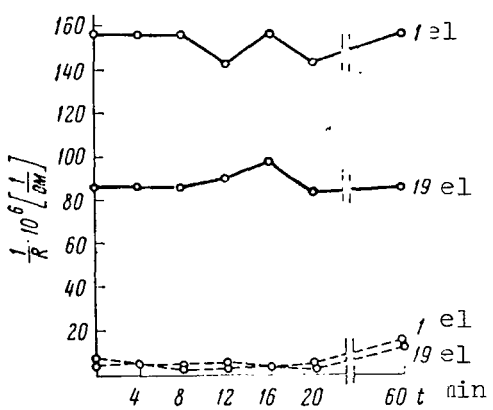

Figure 3. Variation in conductivity between electrodes fed with a voltage and electrodes from which voltages are recorded during prolonged recording. Continuous curves, conductivity for electrodes 1 and 19 when tongue is in contact with palate; broken lines, conductivity for same electrodes without contact between tongue and palate.

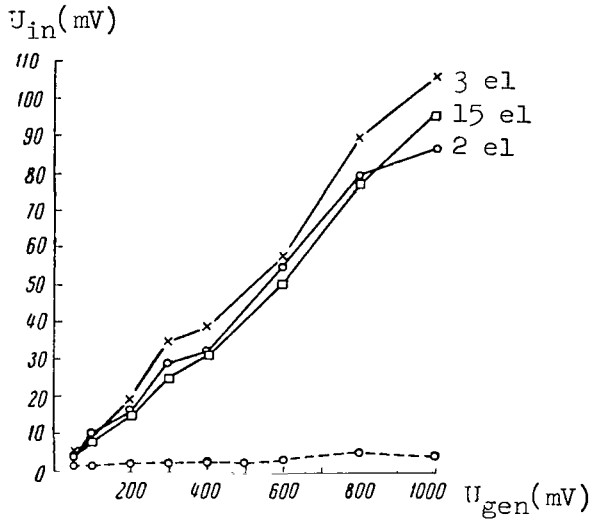

Figure 4. Variation in magnitude of voltage (U_{in}) picked up from different electrodes of sensor as function of voltage fed to sensor (U_{gen}). 3rd, 15th and 2nd el, voltages on electrodes 3, 15 and 2 when tongue touched palate at corresponding points. Broken line shows limiting values of U_{in} on same electrodes.

under these conditions the speaker does not experience any unpleasant sensations due to currents, it became possible to use voltages of the order of 600 - 1,000 mV in future experiments.

To determine the limits within which the useful signals could be varied, the same method was used to determine th intensity of signals at electrode No. 3 when the following sounds were pronounced by various speakers: (t, t', d, d', s, s', z, z', c, ch, n, n', l, l'). A total of four speakers was used. The sounds were pronounced in different phonetic positions associated with two-syllable words, using accented and nonaccented syllables. The voltage at the input to the sensor in all cases was 600 mV. The results obtained showed that:

(a) the average value of the voltage on element No. 3, when these sounds were pronounced, varied in different speakers from 46 - 128 mV;

(b) no substantial difference in the magnitude of the signals was noted when consonants were articulated in accented and nonaccented syllables;

(c) on the average, the magnitude of the signal during the articulation of soft consonants was greater than the magnitude of the signal during the articulation of hard consonants; /567

(d) the magnitude of the signals varied substantially from one pronunciation to another, but was never less than 27 mV.[1]

The noise level measured during the course of the experiment never exceeded 6 mV.

Thus, since the noise level remains relatively stable and substantially less than the possible values of the signals, the automatic detection of signals may be achieved with rather simple engineering means, specifically by means of an amplitude selector with a fixed selection level. Furthermore, since the useful signal indicates the presence or absence of contact between the tongue and the palate, the variation in the absolute values of the signals is not significant.

The most complex conditions for determining the absence of a signal is determining the presence of a slit during the articulation of slit prepalatal sounds s, s', z, z'. To distinguish the palatograms characteristic for this group of sounds from palatogram characteristics for prepalatal sounds, when the tongue touches the palate, points were selected, as shown in figure 1 by Nos. 1 and 2. In the course of preliminary recordings it was established that during the articulation of sounds when the tongue touches the palate, contact of the tongue was recorded at least at one of these points. During the articulation of slit sounds the signal is not fed to the electrodes. Nevertheless, since the width of the slit during the articulation of s and z is several millimeters in size, it was necessary to see whether the articulation of slit sounds is accompanied by induced interference at the electrodes, which would make detection of a slit impossible by automatic means. To verify this, the values of the signals of electrodes Nos. 1 and 2 were measured during the articulation of sounds (s, s', z, z'), as contained in 16 different words. Altogether the sounds were pronounced 144 times by three speakers. In all cases except one the signal on electrodes Nos. 1 and 2 did not exceed the level of noise which takes place when the mouth of the subject is open.

These data show that electrodes placed close to each other do not interact. Consequently, the configuration of the contact zone can be established with a high degree of accuracy.

To determine the optimum carrier frequency, oscillographic recordings were made of signals obtained from the sensor when the frequency of the sensor current was varied. This was accomplished by using a type N-102 oscilloscope equipped with a special amplifier. The oscillograms obtained showed that in the range of frequencies, at least up to 8000 cps, there is no increase in the noise level. Therefore, the sensor could be fed with a frequency of the order of several /568 thousand cycles per second. As a rule we used a frequency of 2,000 cps with a smoothing time constant of the order of 5 msec. Since the rise time of the actual signals, as we see on the oscillogram, is measured by several milliseconds, the application of this time constant does not produce a significant deterioration in the conditions for detecting the signals.

[1]An exception to this was the sound "l," which was not recorded at all at test point.

One of the most complicated problems is to determine the necessary number of electrodes and their arrangement on the surface of the sensor. Obviously in those cases when it is desirable to obtain the most accurate configuration of the contact zone, the number of electrodes must be a maximum. However, under most experimental conditions it is necessary to use only those electrodes which distinguish certain typical palatogram configurations. In particular, when investigating the flow of speech, the measurement of time integrals between signals appearing in different channels of the sensor is meaningful only when the signals are sufficiently reliable signs for the presence of definite phonetic phenomena. The possibility of isolating such signs is not obvious. The data obtained by the method of static palatograms show that the contact zones, when the same sound is produced, vary substantially from one speaker to another (ref. 2) and from one pronunciation to another for the same speaker (ref. 14). Thus, it was necessary to establish whether it is possible to determine some limited collection of points where the presence or absence of a contact will distinguish palatogram patterns characteristic for the articulation of different sounds.

To solve this problem, classical methodology was used to obtain static palatograms of various sounds as pronounced by a speaker. Comparing these palatograms, palate zones were isolated where there is constant contact during articulation of sounds belonging to one group and where contact is absent during articulation of sounds of another group.[1] When sensors were fabricated, the electrodes were located precisely in these zones. Further investigations were carried out by recording dynamic palatograms. The speakers read tables of words in which the sounds occurred in different positions. The tempo of the pronunciation was not controlled and was approximately equal to the average speed with which /569 the text was read. We present data of the graphic processing of the readings.

When sounds s, s', z, z' were pronounced by 3 speakers, the presence of slits was correctly determined for 279 of 282 cases, i.e., a signal was erroneously detected in only 1 percent of cases on a middle electrode (No. 1 or No. 2). When pronouncing palatal sounds (t, t', d, d', l, l', n, n'), the signal on the middle electrodes (or on both) was recorded in 815 of 828 cases.[2] Presence of the signal in element No. 3 in the zone characteristic for articulation of anterolingual sounds was correct for palatal and split sounds.

Furthermore, in a series of cases the presence of contact at the point No. 3 was determined during the articulation of soft postpalatal sounds k', g', x'. However, recordings have shown that during the articulation of these sounds contact at point No. 8 is detected with sufficient reliability. Thus, of the 71 cases in which sounds k', g' and x' were articulated by two speakers, contact at this point always took place. During the articulation of the prepalatal sounds (t, t', d, d', s, s', l, l', z, z', n) by the same speakers, the presence of contact at point No. 8 was not detected at any time (a total of 354 cases). Thus, if we consider the indication of a prepalatal sound to be a complex phenomenon

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[1]Of the 13 cases in which contact at these points was not detected, 11 cases occurred with the same speaker.

[2]Isolating such zones does not satisfy the problem. With static palatograms the sounds are pronounced in definite phonetic positions, and since their number was limited, the pictures vary over a greater range during coherent speech.

(the presence of contact at point No. 3 when there is no contact at point No. 8), the separation of palatograms characteristic for these sounds may be carried out with sufficient reliability.

In the overwhelming majority of cases the articulations of the sound ch displayed a contact at point No. 3. However, the most characteristic indication on the palatogram during the articulation of this sound is the presence of contact at point No. 5. During pronunciation of hard prepalatal sounds (126 cases) contact at this point was not recorded at any time.

Similar data were obtained when signals were automatically detected in the process of articulating prepalatal sounds contained in various words by 5 speakers. The experimental setup used in these experiments is described in the work of Shuplyakov (ref. 11). In these experiments the following facts were established:

(a) The presence of contact at point No. 3 during articulation of t, t', d, d', s, s', z, z', l, l', n, n', c is detected in 99.4 percent of cases;

(b) The presence of contact in at least one of the two points No. 1 or No. 2 during articulation of all these sounds except s, s', z, z', is detected in 97 percent of the cases. Most errors took place during the articulation of l;

(c) The presence of a slit during articulation of s, s', z, z' is detected in more than 99 percent of cases;

(d) Contact at point No. 5 during articulation of c was recorded in 143 of 150 cases when this sound was pronounced. When t, l, l', n were pronounced, /570 contact at this point was not recorded at any time.

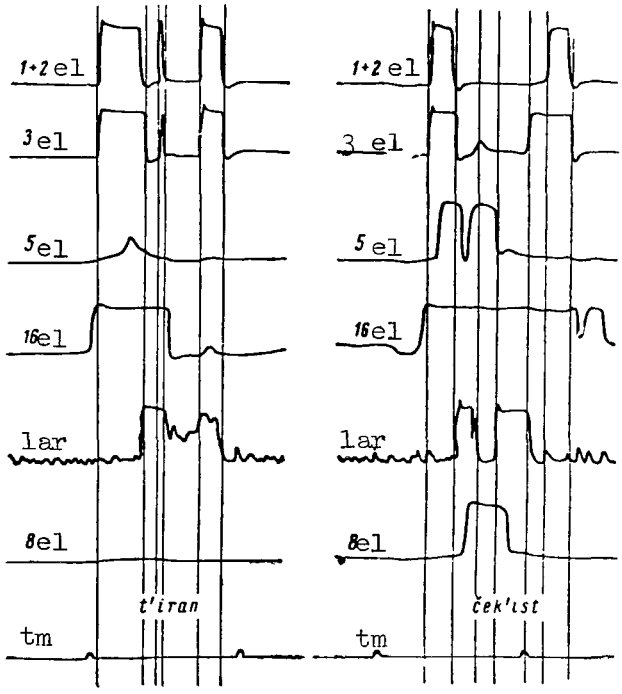
However, during articulation of soft prepalatal sounds (t' and d') contact at point 5 took place in 15 of 242 cases.

The problem of detecting a sign characteristic for articulation of soft prepalatal sounds turned out to be more complicated. It is well known that the soft sounds differ from the hard sounds with the same articulation place by a wider contact zone. With these considerations in mind, electrode No. 11 was chosen as an indication of softness. This electrode was placed slightly above the contact zone characteristic of the articulation of hard sounds (t, d, s, z, c). We found that contact at this point indeed takes place for all cases when the soft sounds (t', d', s', z', n') are articulated. However, in a large number of cases, articulation of the corresponding hard sounds was accompanied by contact at point No. 11. If the electrode is moved farther up so that the hard sounds are not recorded, the recording of soft sounds also becomes unreliable.

It thus becomes impossible to distinguish between palatograms which are characteristic for soft and hard prepalatal sounds on the basis of signal combinations at definite points. In other words, the contact zone during articulation of the sounds is the same in a series of cases. Nevertheless, as we see in the example t and t' (figs. 3a and b), even with the same combination of signals their time relationship during the articulation of hard and soft sounds

is not identical. During the articulation of hard sounds the contact zone becomes narrow at the time when the tongue is separated and an explosion is formed--the contact at point 11 is separated at approximately the same time or a little earlier than the separation at point No. 3. During the articulation of soft sounds contact at point No. 11 is retained during the duration of the explosion. Consequently, the width of the contact zone during the duration of the contact is not significant phonetically and is not controlled with sufficient accuracy during the articulation process. However, at the instant of forming the explosion the contact zone assumes a configuration characteristic of the particular sound (soft or hard). These results agree well with phonetic data, according to which information on softness is contained in the last phase of the consonant and during transition to the subsequent vowel (ref. 3).

A similar situation is observed during articulation of ch and the soft sounds (t' and d'). As we have pointed out, in a series of cases during articulation of (t' and d') there was contact at point No. 5, which is characteristic for articulation of ch. However, towards the end of contact separation at point No. 3 a signal combination is formed which is specifically characteristic of this sound (figs. 5a and b).



a b

Figure 5. Dynamic palatograms for words "t'iran" (a) and "ček'ist" (b). Symbols are the same as in figure 2.

Thus the similarity between the palatograms of different sounds observed during the application of static palatography is to a certain extent an artifact, because in the application of this method the palatogram results /571 from the superposition of contact zones during the period of the pronunciation. If, however, we take into account the variation of the contact zone between the tongue and the palate with time, we can describe the palatogram configurations characteristic for various sounds in a well-defined manner. It is clear that the significant phonetic factor in this case is not the presence of some signal (contact at a definite point), but the realization of some more complex phenomenon.

Thus, the method of dynamic palatography continuously records the position of the tongue during articulation of consonants in the process of coherent speech. The measurements which have been presented show that the noise level during the recording session is quite low, which makes it possible to detect these signals automatically with a sufficient degree of reliability. The number of points which can be recorded simultaneously may be quite large; however, in a series of experiments a relatively small number of points can be used, and the presence and absence of contact at these points may be used to detect substantial phonetic phenomena with a sufficient degree of reliability. Since the application of the method has practically no effect on the intelligibility of speech, the signals obtained during the recording may be used to produce a segmentation of the speech into sections corresponding to definite articulation positions. Sections of the acoustic track obtained in this manner may be used in psychological experiments (ref. 7). In a series of cases when certain indicators are determined, the significant feature is not the presence of signal combinations, but rather their sequence in time. In such cases the automatic device which produces segmentation must process these signals in a certain logical manner.

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AUTOMATIC APPARATUS FOR PRODUCING REVERSIBLE AND REGULATED HYPOTHERMIA FOR POSSIBLE USE UNDER SPACEFLIGHT CONDITIONS

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Artificial hypothermia plays an important role in the many attempts /573 to prevent injury from dangerous space environment. By using deep and reversible cooling of the body under anesthesia, it is possible to lower the metabolism processes and to suppress reactivity to a degree unattainable by other measures. In this case we can expect a decrease in the reaction of an organism to some extremely dangerous effects of space (severe hypoxia, radiation, large G-forces, etc.).

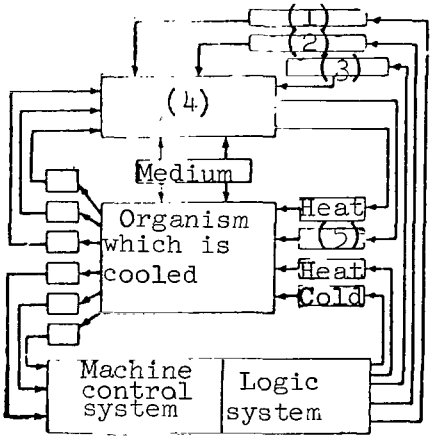
To apply hypothermia under conditions of spaceflight we must place the organism automatically into a state of hypothermia.

From an engineering point of view the design of an automatic machine for deep and reversible cooling of a body is not trivial. The problem is reduced to one rarely encountered in engineering, involving a "struggle" between two automatic machines: the regulating systems of the living organism (thermoregulation) and a technical device which acts externally on the organism through the cooling device (fig. 1). From this point of view the design of an automatic hypothermia machine, which will lower and maintain body temperature in accordance with a fixed program and do so outside the normal biological bounds, is a problem of cybernetics. The problem can be broken down into the following specific aspects.

1. The selection of a system of physical sensors to control the temperature of the internal medium and the operation of basic automatic biological systems.

2. The development of a logic device which will automatically pro- /574 cess the readings of the sensors to give preference to the technical automatic systems during the period of hypothermia and to biological automatic systems during all other intervals of time.

3. The development of a programming unit to permit the experimentator or physician to assign and control the duration and mode of the hypothermia process.



(1) Anesthesia
(2) Stimulation
(3) Main anesthesia
(4) Biological control system (Brain)
(5) Cooling

Figure 1. Operation of automatic machine for reversible hypothermia.

4. The development of a control system to supply the components of the gaseous mixture to the mask and to control the cooling or heating liquid flowing into the thermostatically controlled suit. From the scientific and engineering point of view the third and fourth problems are not new; they are therefore not considered at this time.

Our own experiments and the data published in the literature have shown that the optimum limits of physiological function during the cooling of anesthesized animals are given by the following values: rectal temperature must not fall below $+22^{\circ}$ (x_1) and must not rise above $+26^{\circ}$ (x_2), skin temperature must not drop below $+3^{\circ}$ (x_3) and must not rise above $+14^{\circ}$ (x_4), respiration depth must not increase by more than a factor of 10 compared with the initial level (x_5), change in the respiration rate must be within the range of 6-60 per min (x_6), arterial pressure must not drop by more than 5 times (x_7) and there must be no motor activity (x_8).

Within these limits the physiological functions are sufficiently depressed, and there is, at the same time, no danger of the development of pathological disorders.

These physiological parameters were converted into electric pulses /575 which, at the output of the sensors, were proportional to the measured quantities within the assigned limits. The readings of all sensors were transformed to the variation of eight binary variables. Each output variable could

have only two values: the presence of a signal, if the measured quantity was above or below the assigned limits (I), or the absence of a signal (0), if the quantity was within the permissible limits.

The logic circuits show the necessary reactions of the automatic setup: the switching on of cooling and heating (table 1), the supply of respiratory stimulant, the supply of normal anesthetic mixture and use of forced anesthesia (table 2).

The logic circuits contain a series of different physiological function parameter combinations. Among the possible combinations x_1, x_2, x_3, x_4 (table 1), nine have physiological meaning. Combination No. 1 represents the optimum state of hypothermia within the assigned limits: heating and cooling are absent. Combinations No. 3 and 6 signal the danger of freezing integuments. In this case the automatic apparatus is signalled to cease cooling and to turn on heating or to discontinue both: when the rectal temperature is 26°C, the skin can be heated by the redistribution of blood flow and the influx of heat from the deeper parts of the organism.

With combinations No. 5, 7 and 9 the rectal temperature falls below the specified limits; heating is turned on. If, under these conditions, the skin temperature is above 14°C (combination No. 7), external heating is not required; the temperature of the body will rise due to the flow of warm blood from the surface of the body. Combinations No. 2 and 8 show that the rise in skin temperature is above the specified limits; it is necessary to apply external cooling (combination No. 8) or to turn on cooling and heating; the skin temperature will then drop due to the redistribution of the blood (combination No. 2). Combinations No. 4 and 8--the rise in rectal and skin temperature--require that the cooling device be turned on. /576

In the network controlling the supply of gaseous mixture a large number of combinations (16) have physiological meaning (table 2).

Combination No. 1 represents to optimum state of hypothermia during narcosis with a satisfactory depth and rate of respiration, arterial pressure level and the absence of motion and shivering.

TABLE 1. NETWORK FOR CONTROLLING BODY TEMPERATURE.

Combination No.	x_1	x_2	x_3	x_4	Cooling	Heating	Combination No.	x_1	x_2	x_3	x_4	Cooling	Heating
1	0	0	0	0	0	0	6	0	1	1	0	0	1
2	0	0	0	1	0	0	7	1	0	0	1	0	0
3	0	0	1	0	0	1	8	0	1	0	1	1	0
4	0	1	0	0	1	0	9	1	0	1	0	0	1
5	1	0	0	0	0	1							

TABLE 2. NETWORK FOR CONTROLLING SUPPLY OF GASEOUS MIXTURE.

Combination No.	x_5	x_6	x_7	x_8	O_2	80%-N_2O 20%-O_2	N_2O	Combination No.	x_5	x_6	x_7	x_8	O_2	80%-N_2O 20%-O_2	N_2O
1	0	0	0	0	0	1	0	9	1	0	0	1	1	0	0
2	0	0	0	1	0	0	1	10	0	1	0	1	0	0	1
3	0	0	1	0	1	0	0	11	1	0	1	0	1	0	0
4	0	1	0	0	0	1	0	12	1	1	0	1	1	0	0
5	1	0	0	0	1	0	0	13	1	0	1	1	1	0	0
6	0	0	1	1	0	0	1	14	0	1	1	1	0	0	1
7	0	1	1	0	0	1	0	15	1	1	1	0	1	0	0
8	1	1	0	0	1	0	0	16	1	1	1	1	1	0	0

Combinations No. 3, 5, 8, 9, 11, 12, 13, 15 and 16 may be characterized as the state of an organism with insufficient respiration. The countermeasures in this case must consist of immediate respiration stimulation. The normalization of respiration in these conditions should naturally lead to the restoration of arterial pressure to its normal values. If the motions were of an asphyctic origin, they should disappear. If motions remain, then combinations will occur which will point to the awakening of the animal, and the reactions of the machine will lead to deeper narcosis. An exception is combination No. 3. In this combination the depth and frequency of respiration remain in the normal limits, but the arterial pressure is below the critical level. This state is highly improbable, because an overdose of anesthesia will first be manifested as a disorder in respiration. Nevertheless, this combination is still provided for: the countermeasure of the machine will be to supply pure oxygen or carbogen.

Combinations No. 2, 4, 6, 7, 10 and 14 are characterized by a normal or deep and frequent "labored" breathing, movements are present or absent. These combinations correspond to the awakening of the animal. The response of the system when motion is present must be to increase anesthesia--supply 100 percent nitrous oxide (Shch-1). /577

The disjunctive forms of the switching functions before minimization have the following form for the network which controls the temperature

$$Q(x) = \bar{x}_1 x_2 \bar{x}_3 \bar{x}_4 \vee \bar{x}_1 x_2 \bar{x}_3 x_4;$$
$$P(x) = \bar{x}_1 \bar{x}_2 x_3 \bar{x}_4 \vee x_1 \bar{x}_2 \bar{x}_3 \bar{x}_4 \vee \bar{x}_1 x_2 x_3 \bar{x}_4 \vee x_1 \bar{x}_2 x_3 \bar{x}_4, \quad (I)$$

where P(x) is the supply of heat;
Q(x) is the supply of cold.

For the network which controls the gaseous mixture we have

$$\begin{aligned} B(x) &= \bar{x}_5\bar{x}_6x_7\bar{x}_8 \vee x_5\bar{x}_6\bar{x}_7\bar{x}_8 \vee x_5x_6\bar{x}_7\bar{x}_8 \vee x_5\bar{x}_6\bar{x}_7x_8 \vee x_5\bar{x}_6x_7\bar{x}_8 \vee \\ &\vee x_5x_6\bar{x}_7x_8 \vee x_5\bar{x}_6x_7x_8 \vee x_5x_6x_7\bar{x}_8 \vee x_5x_6x_7x_8 \\ N(x) &= \bar{x}_5\bar{x}_6\bar{x}_7\bar{x}_8 \vee \bar{x}_5x_6\bar{x}_7\bar{x}_8 \vee \bar{x}_5x_6x_7\bar{x}_8 \\ \text{Shch}(x) &= \bar{x}_5\bar{x}_6\bar{x}_7x_8 \vee \bar{x}_5\bar{x}_6x_7x_8 \vee \bar{x}_5x_6\bar{x}_7x_8 \vee \bar{x}_5x_6x_7x_8, \end{aligned} \tag{II}$$

where B(x) is the supply of stimulant;
N(x) is the supply of anesthesia;
Shch(x) is the supply of increased anesthesia.

However, expressions (I) and (II) are not optimum. It can be shown that the values of the output variables can be obtained with much simpler combinations of the input variables. Without presenting the cumbersome step-by-step analysis, we present only the final equations.

$$\begin{aligned} Q(x) &= \bar{x}_1x_2\bar{x}_3 \\ P(x) &= \bar{x}_1x_3 \vee x_1\bar{x}_2 \\ B(x) &= \bar{x}_5\bar{x}_6x_7\bar{x}_8 \vee x_5 \\ N(x) &= \bar{x}_5\bar{x}_6\bar{x}_7\bar{x}_8 \vee \bar{x}_5x_6\bar{x}_8 \\ \text{Shch}(x) &= \bar{x}_5x_8. \end{aligned} \tag{III}$$

The block diagram of the control system (fig. 2) was constructed by minimizing expressions (III). At the present time we have developed three models of the automatic hypothermia machine. Below we describe the engineering aspects of model No. III.

In the network for the temperature control the sensors consist of thermistors. The rectal temperature is measured with the T86 3M thermistor, while the skin temperature is measured with the KMT-12 thermistor. Each thermistor is connected in an arm of a bridge, while the opposite arm of the bridge has the potentiometer for controlling the assigned temperature level. One of the diagonals of the bridge is fed with 7V dc. The voltage /578
imbalance is amplified and fed to a limiter-shaper with a relay output. Thus, two circuits shape 4 binary variables x_1 - 4. Each variable takes on the value of unity if the upper or lower level is exceeded and assumes a value of zero if it is within the limits of the assigned level.

The network for the control of the gaseous mixture contains a rheostat sensor for the respiration depth, a contact sensor for motor activity and respiration rate and a compensating sensor for blood pressure. All readings of the sensor are reduced to binary variables. For conversion to the binary

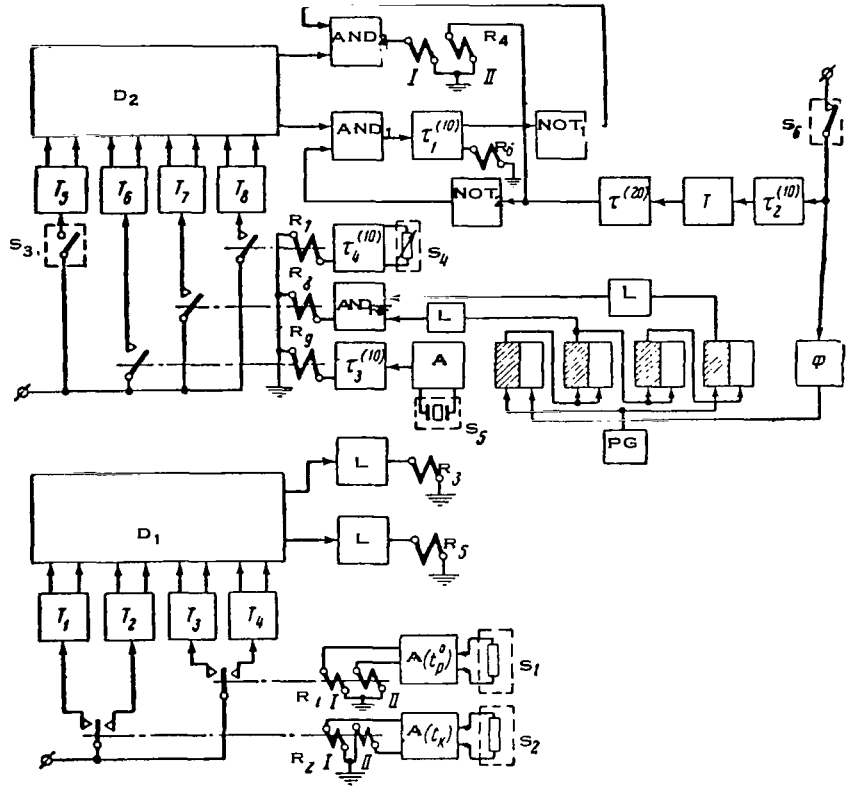


Figure 2. Block diagram of automatic equipment for hypothermia. D, Decoder; S, Sensors; A, Amplifier; PG, Pulse Generator; T, Trigger; T_1 - T_8, Schmidt Triggers; R, Relays; t_p, rectal temperature; t_k, skin temperature; AND_{rf}, subscript: respiration frequency.

variables from the respiration rate sensors, a three-stage binary counter, a pulse generator and a comparison circuit are used.

The basic unit of the logic circuits in both networks are the decoders with four inputs followed by assembly networks. /579

The application of a decoder makes the logic part (L) of the circuit simpler and more reliable. The feature which is very important when experiments are run is that the output part of the logic circuit becomes more flexible because the switching of the actuating organs can be changed quite easily. The input units of the coder use Schmidt triggers which have one stable position. When

the signal is applied the trigger changes its position, and when the signal is removed it returns to its initial state.

The logic functions were realized by using the standard logic circuits of the AND, OR, NOT type. Semiconductors were used as basic elements to obtain a reliable and compact construction with minimum power consumption.

The actuating elements consist of electromagnetic relays whose operating windings are connected to the output of the logic circuit. A device is provided which checks the proper operation of the logical part of this circuit. This device consists of (1) a wafer switch which switches the circuit into the test position; (2) an assembly field where the switches are used to select some combination of signals at the output, and (3) signal lamps lit to show that the circuits are operating properly.

In spite of the complexity of the automatic machine for hypothermia, by using solid state components--transistors, germanium diodes--it was possible to make the equipment sufficiently portable to be used aboard a spacecraft.

We should point out that during the operation of the automatic device the "machine" control is combined and alternated with the organism control of the physiological functions. This also gives the automatic setup a cybernetic nature.

The overall operating characteristics of the device may be presented in the form of a relative (in percent) number of combinations of physiological parameters, which signal that the organism is in danger during the period of hypothermia (freezing of integuments, low body temperature, nonuniform cooling of the tissues, suppression of respiration and lowering of the arterial pressure below the critical level). Such combinations constitute 66 percent. Correspondingly, the largest number of reactions by the automatic device (71 percent) are directed to remove the subject from this dangerous state.

Thus, under conditions of hypothermia under anesthesia, the "organism" control of functions is replaced to a certain extent by the "machine" control designed to maintain the functions of an organism within assigned safe limits.

Considering the wide application of artificial hypothermia in surgical /580
clinics and the experience of producing the automatic systems described above (even though very primitive), we can consider the application of our automatic machine in surgical clinics for narcosis and hypothermia. Experiments conducted with 16 dogs, whose body temperature was brought down to 22-25°C, have shown that the automatic equipment operates in a stable fashion. It cooled the body to an assigned level, maintained the assigned level of hypothermia and the state of narcosis for different periods of time (up to 24 hours) and brought the animals out of this state.

The automatic equipment for hypothermia turned out to be quite reliable. During a 120-hour exploitation of a test subject under conditions of a physiological laboratory not a single failure was observed caused by component failure in the automatic equipment.

It is expedient to continue the work we have started in several directions: to improve the sensitive elements, increase the quantity of information fed to the automatic device (biopotentials of the brain and of the heart) and impart the qualities of self-learning and self-organization to the logic system and the control system.

METHOD OF RECORDING ACTION CURRENTS IN AUTONOMIC NERVES UNDER CHRONIC (CONTINUOUS) EXPERIMENTAL CONDITIONS

A. D. Nozdrachev

When the interrelation of various organs and systems are investigated under conditions simulating spaceflight, one of the basic requirements is to carry out observations during chronic experimental conditions. /581

The present method of recording action currents in different parts of the autonomic nervous system usually involves the short-term experiment. In addition to the fact that the inherent short duration of the short-term experiment sometimes makes it impossible to obtain complete results, this method is also associated with a series of disadvantages. A need has arisen, therefore, for the development of a new method which permits the recording of activity currents in the same nervous structures, but under chronic experimental conditions. The problem was simplified because the published literature contains information on the possibility of implanting electrodes next to the nerve conductors (refs. 1-3).

We developed a method of implanting electrodes into the splanchnic and sinus nerves of the preganglionic and postganglionic branches of the caudal mesenteric ganglion. Before describing the operative approach and the implantation process, we shall briefly consider the construction of the electrodes.

The activity currents are measured by means of a platinum wire with a cross section of 0.1 mm^2 and a length of 10 mm. The flexible conductor consists of a copper wire (MGV-0.1 x 15) with a polyethylene insulation. The strands of the conductor are slightly pulled out of the insulating tube and some of them (5-7 pieces) are cut off. The remaining strands are carefully soldered to the platinum electrode. After this, the insulating tube is drawn over the solder joint and its edges are trimmed with a heated needle. The point where the insulation joins the electrode is additionally covered with fingernail polish.

The next stage consists of preparing the capsule, which contains /582 the finished electrode and into which the nerve column is implanted during the operation. The capsule consists of a plexiglas plate (2 x 7 x 20 mm; fig. 1, 7), one of whose ends contains a groove for a teflon or polyethylene strip. Two longitudinal grooves are made by means of a dentist's drill in the plexiglas plate at a distance of 3-4 mm from each other. One end of the groove bears against the teflon plate (6), which also contains a thin slit. The groove is used for the flexible conductors (5), while the slit in the teflon strip is used for the platinum electrodes (4) leading to the nerve.

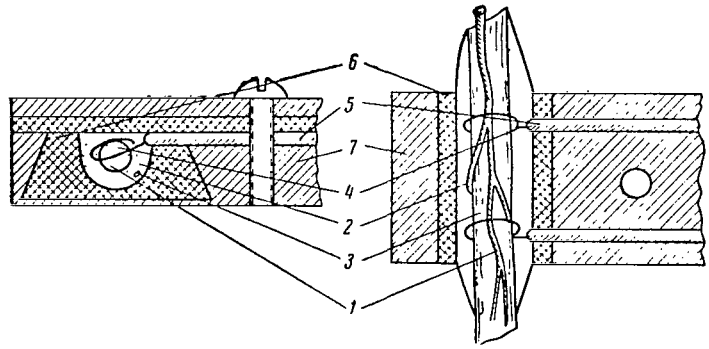


Figure 1. Diagram of position of electrode and of nerve in capsule. 1, Nerve stem; 2, coupling of connective tissue; 3, vessels; 4, platinum electrode; 5, flexible conductor; 6, teflon bed; 7, plexiglas base.

After the conductors with the electrodes are placed into the grooves, they are filled with liquid plexiglas (solution of plexiglas and chloroform) and covered with a thin plate and placed into a vise until dry. This relatively simple operation provides a durable bond between the conductor leads and the capsule.

The remaining part (the teflon strip with the depression for the nerve) is also covered with a teflon plate. The assembly constructed in this manner is covered with a removable plexiglas securing plate. Two threaded holes are drilled in the capsule for the screws holding the removable cover and the teflon plate. The teflon bed together with the plexiglas base provide for the reliable electric insulation for the region containing the nerve and the implanted electrodes. It is known that the nerve does not have the same degree of contact tolerance for different insulating materials. According to our observations, teflon and polyethylene were most suitable.

During a series of preliminary experiments associated with the im- /583
plantation of electrodes into the preganglionic and postganglionic branches of the caudal mesenteric ganglion and into the splanchnic nerve, the dogs had a tendency to pull out or bite off the conductors introduced under their skin. Therefore, a special collector was designed which was implanted into the small of the back. The collector (fig. 2) consists of a plexiglas cylinder with a movable nut (1, 2), a transverse plate with an opening (3) corresponding to the diameter of the flexible conductors. The plate is cemented to the end of the cylinder. At the other end a standard connector is secured by means of screws (in our case a five-pin connector; 4).

The completed capsule and collector are washed, treated with alcohol and stored before use in a physiological solution containing antibiotics. It is undesirable to store the capsule and collector in alcohol, because this produces a deformation of the capsule.

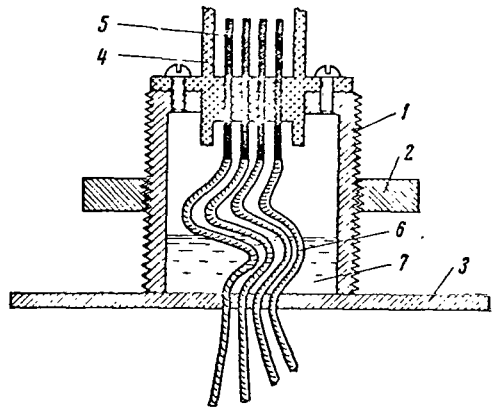


Figure 2. Diagram of collector. 1, Plexiglas tube; 2, movable nut; 3, transverse plate; 4, connector; 5, pins of connectors; 6, flexible conductor; 7, paraffin.

Before the operation is started, the screws holding the upper removable plate of the capsule are removed. The ends of the electrode are bent like fishhooks by means of optic anatomic pincers and are threaded with a very fine silk thread. The collector coupling is also unscrewed and left in this position until the operation is completed.

When the electrodes are implanted into the preganglionic and postganglionic branches of the caudal mesenteric ganglion and into the splanchnic nerve at the level of the transversocostal processes III-IV of the lumbar vertebra, a trocar is introduced. Its tube remains until the operation is completed. Then a laparotomy is performed along the white line, and the caudal mesenteric ganglion with its branches is localized. The nerve stems are prepared so that the blood vessel supply is not injured. On the postganglionic stem (n. /584 colonicus) it is usually possible to see not only the appropriate branches, but also the vasa nervorum. If these vessels are damaged during the preparatory process or when the electrode is introduced, the nerve will inevitably die and the entire operation will be unsuccessful.

The nerve stem is then pierced with an ocular piercing needle connected by the silk thread to the platinum electrode. We had to change from simple insertion of an electrode, because it is difficult to pierce the nerve stem with the thin soft platinum wire. Repeated efforts at piercing produce excess injury to the stem. The second electrode is introduced at a distance of 4 mm from the first. After this both silk threads are tightened and the nerve is secured to the platinum electrode. The ends of the electrodes are bent, the silk threads are cut off and the nerve with the electrodes is placed into the teflon bed. The teflon strip and the removable fixing plate are placed on the top, and the latter is secured with two screws. The capsules must be designed so that the nerve lies freely in the teflon bed. If the nerve is compressed, atrophy is inevitably produced. The capsule is covered with an omentum and is fixed to the abdominal wall by several sutures.

The same operation was performed with the preganglionic stem.

At the point where the capsule is attached, the neutral electrode is suspended. This electrode consists of a multistrand wire in a polyethylene cover. The end of the wire is tinned. This electrode is used to ground the animal.

All flexible conductors are then combined and carried out to the loin through the trocar tube introduced before the operation. The abdominal cavity is sutured. Penicillin is introduced along the suture into the incision.

The second phase of the operation is to implant the collector. For this purpose a deep sagittal incision 4-5 cm long is made at the loin next to the trocar tube, which is still in place. The muscles adjoining the transverso-costal processes of the vertebra are separated, the trocar tube is removed and the free conductors are passed through the openings in the lower plate of the collector. The collector is then inserted into the incision so that its plate is on the transversocostal processes, while the side edges fit under the myo-genic layer. In this manner the collector is held securely by the tissues. The muscles are sutured. A purse-string suture is made on the skin.

The exposed conductors are soldered to the contacts of the connector, the cylindrical collector cavity is dried with a tampon and filled with melted paraffin. The latter step is absolutely necessary to prevent the flow /585 of inflammatory exudate through the holes of the plate and the shorting of the connector contacts.

A similar operation was conducted to implant electrodes into the splanchnic nerve. To provide for operative access an additional incision is made along the vertebra arch.

When electrodes are implanted into the sinus nerve, the carotid artery is exposed by the middle incision and the subsequent blunt preparatory period. The sinus nerve is localized and prepared. The remainder of the procedure for implanting the electrodes into the nerves does not differ substantially from the one described previously. By means of bullet forceps the flexible con-ductors are passed under the skin to the cranium and are here soldered to the connector attached to the sagittal crest. The wound is sutured in the conven-tional manner.

When the activity currents are recorded in the days immediately following the operation, interference may result due to the formation of inflammation around the capsule. Most of this interference disappears as the auxiliary electrode becomes fully grounded.

After several days a strong connective tissue covering is formed which covers and holds the capsule in place. The formation of the sheath together with the correct installation of the electrodes assures that the character of action currents will remain constant over a long period of time. The analysis of neurograms of our dogs for a period of ten months after the implantation of electrodes showed that there was no variation in the nature of the action currents.

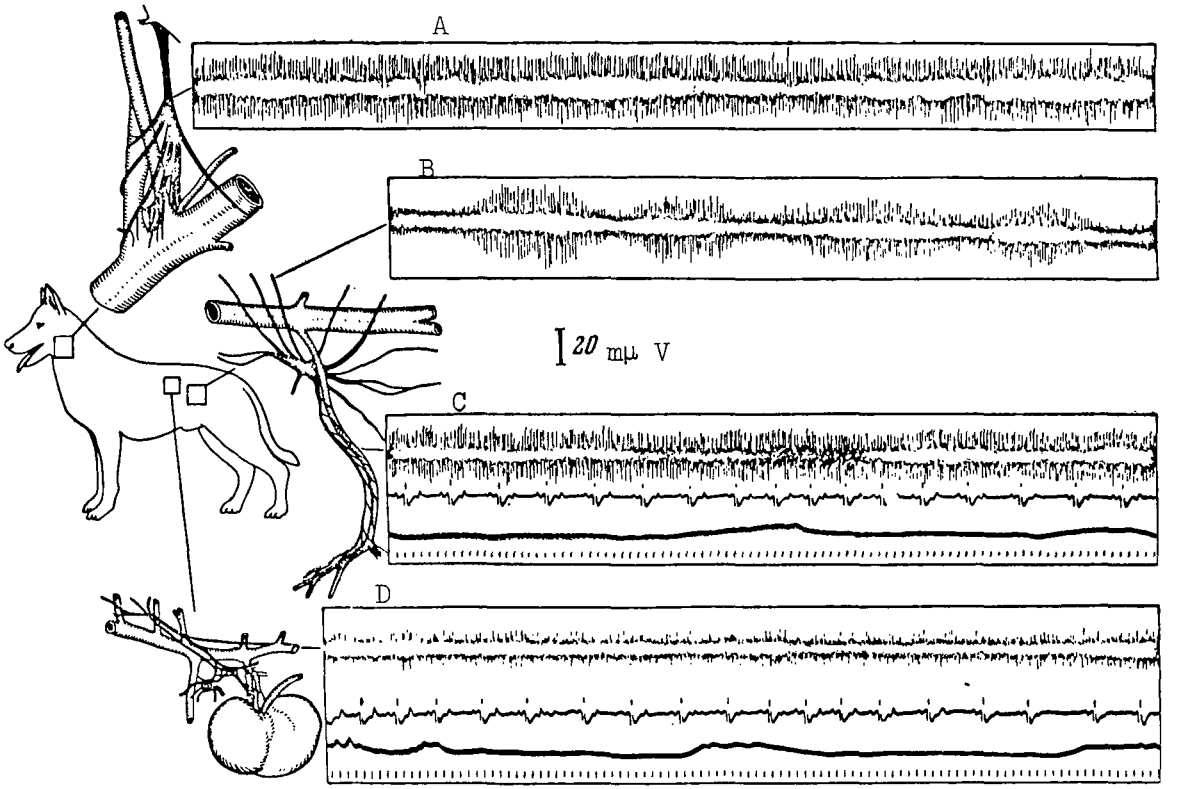


Figure 3. Action currents in autonomic nerves 10 months after electrode implantation. A, Sinus nerve; B, preganglionic branch; C, postganglionic branch of caudal mesenteric ganglion; D, splanchnic. Time index 10 cps.

In addition, the connective tissue proliferates and forms a sheath with blood vessels around the nerve, rigidly fixing the capsule.

The method was verified by conducting brief experiments with oscillograph recordings on the same nerve formations. A comparison of the characteristics showed that they are the same both for the brief and the chronically studied experiments.

Figure 3 shows the action currents from the preganglionic and postganglio branches of the caudal mesenteric ganglion and the sinus and splanchnic nerves ten months after the implantation of the electrodes.

The proposed methodology makes it possible to record action currents from autonomic nerves over a prolonged period of time.

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METHOD FOR RECORDING VENOUS OUTFLOW IN CEREBRAL VESSELS OF ANIMALS DURING EXPOSURE TO ACCELERATION

V. Ya. Klimovitskiy and V. F. Nikolayev

The investigation of the effect of acceleration on blood circulation /587 in the brain of man and animal is one of the important problems in biology and medicine associated with providing spaceflight safety. We can assume that the venous cerebral outflow is quite sensitive to the action of acceleration. It has been established that the blood filling of the brain is sensitive to very small changes in gravitation (ref. 4). The state of the venous outflow and the filling of the brain's venous system are important hemodynamic factors which determine the condition of intracranial blood circulation (refs. 2 and 3). Clarification of the compensating possibilities of the cerebral vascular system, study of the dynamics of cerebral circulation at the instant of and after the action of G-forces and establishment of methods to prevent the disruption of cerebral circulation must become the subject of special investigations.

We developed a method which records the blood flow rate in large surface cerebral veins of experimental animals under actual conditions of flight. The primary transducer has the form of a heat exchange sensor (thermal sensor). All methods which measure the thermal conductivity of living tissues, which indirectly characterizes the blood flow rate in the test subject, have a series of limitations. The principal one of these is the impossibility of obtaining absolute values of blood flow rate because of the distortions introduced by the measuring equipment when rapid processes are recorded (refs. 14 and 18). Nevertheless, thermal sensors (fig. 1) are widely used in the investigation of blood flow rate dynamics when it is necessary to conduct a prolonged continuous recording of blood flow rate in small volumes of tissues or in vessels of small diameter. The theoretical premises for applying the heat exchange sen- /588 sors of blood circulation rate are covered in the works of a series of authors (refs. 6-8, 11, 13, 15, 16 and 20).

In our methodology the transmitting and receiving elements of the sensor are placed on both sides of the vein or of the venous sinus on the surface of the brain. Thus heat exchange takes place through the blood vessel. Unlike the technique of recording the blood flow rate by means of a differential thermal element (refs. 1 and 5), we used only one semiconductor thermistor. To isolate the temperature as a function of thermal conductivity in the investigated region from the thermal noise of the living medium (the oscillations of heat production by the brain tissues), we applied the method of the nonstationary thermal field (refs. 9, 10 and 12). However, instead of a three-stage heating, due to certain theoretical consideration, we applied a one-stage heating. A thermal

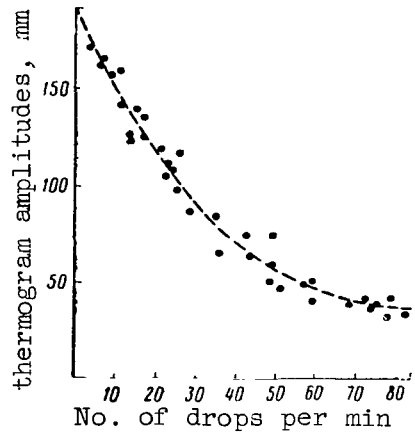


Figure 1. Calibration curve for thermal sensors.

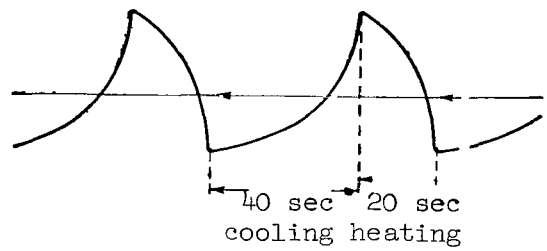


Figure 2. Temperature variation cycle in thermal sensor. Temperature rise during heating is approximately 1°C.

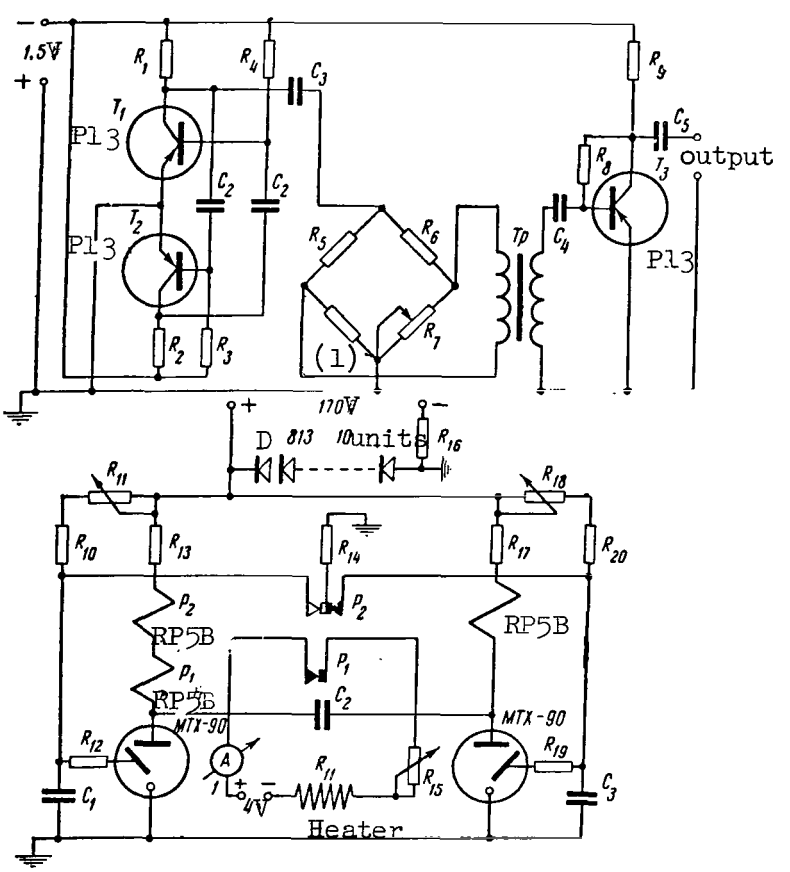
impulse of fixed flux and fixed time was developed by the heater and was recorded by means of a thermistor on the other side of the blood vessel. Thus the temperature maximum of each thermal impulse was inversely proportional to the total blood flow in the investigated blood vessel during the period of one cycle. The curves showing the variation in the temperature amplitudes of a thermogram as a function of blood flow have an exponential nature. The sensors were calibrated by using the femoral vein of a cat in a short-term experiment. Thus the proposed methodology makes it possible to follow the dynamics of blood flow, but it does not obtain the absolute value of blood flow rate. The operation of the sensor was cyclic. The cycle included the heating period (20 sec) and the cooling period (40 sec) (fig. 2). To record rapid processes the cycle period can be reduced by a factor of 5-6. It is not desirable to decrease the time too much because of the relatively high time constant of the sensor.

All measurements were carried out in the chronic experiments using rabbits. The sensor was installed on the large surface veins before they recess into the front longitudinal sinus or directly on the front longitudinal sinus. The trepanation opening with a diameter of 8 mm was made in the parietal region. The hard meninx was removed. The bone defect was hermetically replaced by the

sensor case. The rabbits were used in the experiments 5-7 days after the operation and were still suitable for experimentation after a month or more /589 following the operation. The condition of the animals during all this time was quite satisfactory.

The device for recording the blood flow (fig. 3) consists of am audio generator, a bridge with a thermistor in one arm and an amplifier. The heaters are connected and disconnected by means of a time relay using two MTX-90 thyratrons. The bridge is powered by an audio frequency generator. The latter consists of a multivibrator utilizing P13 transistors.

From the bridge the signal was fed to an amplifier, using a P13B transistor, and then to the input of a portable tape recorder. The bridge was operated with a small controllable imbalance to provide recording in the range of possible biological temperature oscillations. The entire device was powered from a series of batteries for the continuous operation of the device for a period of 10 hours. The weight of the device with the power source is 3.2 kg and with the tape recorder 5.7 kg. The device is designed for installation on a laboratory centrifuge. In order to process the thermograms they were copied from the magnetic tape by a type N-370 or EPP-09 recorder.



(1) Thermistor

Figure 3. Circuit of generator amplifier and time relay.

We had access to a centrifuge with a constant speed of 135 rpm and a radius of 0.8 m. We used this centrifuge to conduct experiments with rabbits for the purpose of clarifying the operational properties of the device and for obtaining preliminary data on the effect of G-forces on the venous outflow in the cerebral blood vessels. It was established that the device operates stably with an 8 G-force and does not displace the time cycle by more than a few tenths of a second per cycle (after 200 hours of continuous thyratron operation).

The rabbit was fixed in a prone horizontal position, with the head toward the center. Under these conditions the G-force at the level of the head was approximately 5, at the thorax it was 8 and at the pelvis 10. The head of the animal was secured. Figures 4 and 5 show the thermograms obtained in one /590 experiment. During the first day of the experiments the rabbit was subjected to the action of the centrifuge 4 times, 30 sec each time (from the time the motor was turned on to the time it was turned off, i.e., including an acceleration time of 3 sec and excluding the deceleration period of 7 sec). The interval between tests was 20 to 30 min.

During the first runs of the centrifuge the increase in the venous outflow was clearly defined during the period of the thermal cycle, which took place every rotation cycle. There was some decrease during the next two cycles and the phenomena were accompanied by some decrease in the cerebral temperature.

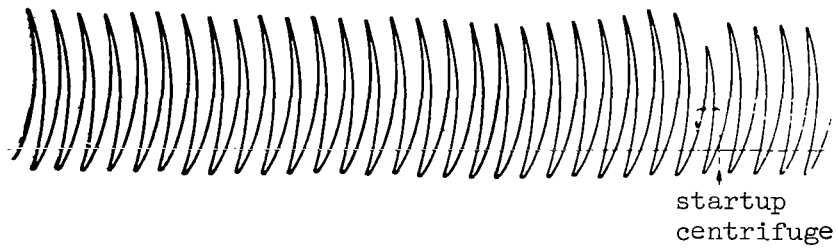


Figure 4. Thermogram taken when rabbit was rotated during first day of experiment. Second startup of centrifuge.

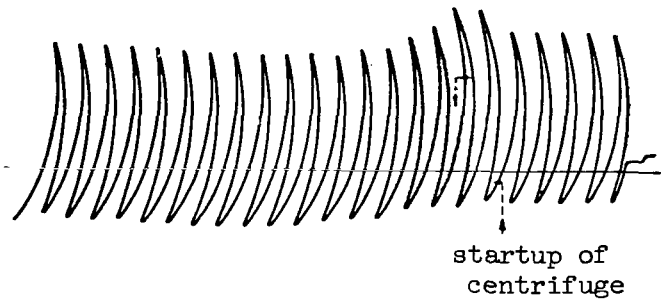


Figure 5. Thermogram taken during second day of experiment. Seventh startup of centrifuge.

During subsequent tests the decrease in the venous outflow and the drop in temperature was greater, while the increase in blood flow rate during the first cycle disappeared. During the fourth experiment on the centrifuge there /591 were only decrease in the blood flow rate and a substantial temperature drop with subsequent restoration.

On the second and third day of the experiment under the same conditions we observed only a decrease in the venous outflow, beginning with the first cycle. The load was then increased to 1-1.5 min of rotation. This did not change the picture substantially.

During the first day of experiment following a one-day interruption we observed that when the centrifuge was started the blood flow did not change during the first minute; then the effect already noted occurred, reducing the blood flow in the investigated vessel. Subsequently, as in the preceding cases, the decrease in the efflux was already observed during the first minute.

From the preliminary observations we can propose that the main result of the acceleration effect in the direction "head-pelvis" on the venous outflow is the decrease of blood flow in the large veins and sinuses of the animal's brain.

The difference in blood flow reactions with completely standard physical conditions, under which the centrifugal force was applied, the regular variation of reactions during a series of subsequent loads--shows that physiological /592 mechanisms participate in these processes. We assume that their role is reduced to providing the stable blood supply to the brain--the increase in the effect of blood flow reduction during repeated multiple exposures may be explained as a gradual depletion of compensation possibilities.

The latter resembles in its nature the similar reaction phase on blood flow when 5-10 percent carbon dioxide is introduced into the inhaled air. In all probability this analogy is quite substantial, because during longitudinal G-forces some anematization of the brain is possible as well as local hypercapnia.

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COURSE AND SEQUELAE OF BODY COOLING BY CONTROLLED HYPOTHERMIA

G. N. Il'yutkin

A large circle of investigators has been attracted to the problem of /593 hypothermia. Hypothermia has found application in clinical and experimental surgery.

The state of hypothermia increases the resistance to oxygen deficiencies, poisons, various types of infections, radiation and the effect of accelerations and associated G-forces and traumatic shock and trauma pertaining to burns. These data permit us to look on hypothermia as a possible method of increasing the stability of the organism against unfavorable effects produced on the organism of a cosmonaut during interplanetary flight.

However, the use of hypothermia for these purposes requires special equipment to bring the organism into a state of hypothermia and to remove it from this state by means of automatic devices. With these considerations in mind, a cybernetic apparatus was built to produce reversible and controlled hypothermia. The present article presents the results of experiments with animals cooled by means of this apparatus.

Forty-six experiments were carried out with dogs. The animals were given a morphine injection computed on the basis of 10 mg of dry substance per 1 kg of weight. After the animals became drowsy they were outfitted with a mask through which an anesthetic was applied: nitrous oxide with oxygen in the ratio of 4:1-5:1. Sleep was induced after 1-3 min.

The operational cooling device is shown in figure 1. It consists of the FAK-07 refrigeration assembly, with evaporators submerged in a tank filled with a solution of ethylene glycol. During the operation of the equipment the temperature of the solution is decreased to -25°C. The cooled solution is pumped through a solenoidal valve into a thermal suit and then returned to the tank for cooling. The temperature of the thermal suit is approximately -10°C. To heat the animal, a solution of ethylene glycol heated to +45°C was fed from a thermostat into the thermal suit. The motion of the cold or hot liquid in the thermal suit was controlled by means of solenoidal valves controlled automatically or /594 manually from the control point.

Of 46 experiments 31 were carried out with manual control and 15 with automatic control.

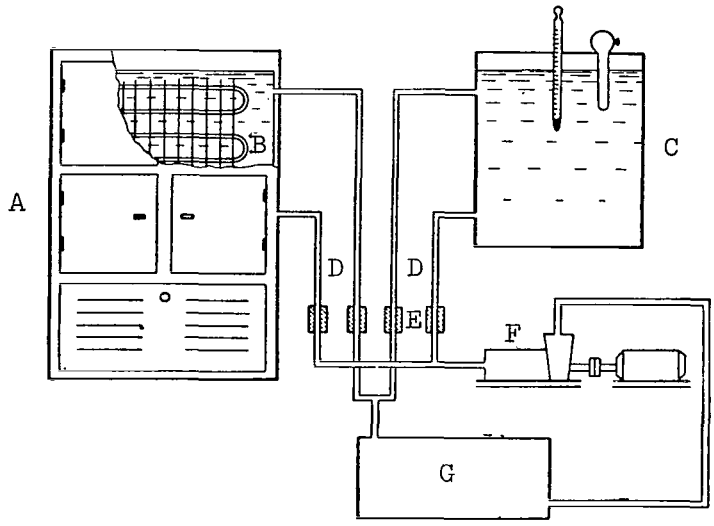


Figure 1. Cooling equipment. A, cooling chamber; B, tank with ethylene glycol solution; C, thermostat with ethylene glycol solution; D, piping system; E, solenoidal valves; F, pump with electric motor; G, thermal suit.

Thirteen dogs were used to carry out repetitive experiments with hypothermia. In eight dogs the experiments were repeated three to six times.

Before the experiment, during the time of hypothermia and at various periods after hypothermia, clinical and laboratory investigations of the animals were carried out: weight and temperature of the body were determined; the quantity of positive blood elements and of the hemoglobin, erythrocyte sedimentation rate, pulse rate and respiration rate and pulmonary ventilation were determined. The rectal temperature was measured, and the arterial pressure and respiration were recorded on a kymograph. The sequence of manipulations was as follows: after the dog was anesthetized, the femoral artery was isolated for the purpose of recording blood pressure, a rheostat sensor was placed on the dog's chest to record the depth of respiration and a contact sensor was placed to record the respiration rate. The thermal suit was put on, and two thermistors were inserted into the rectum: one for visual observation of body temperature, and the other for feeding an electric pulse into an automatic device. These thermistors were previously graduated. The thermistor for measuring skin temperatures was /595 then attached. The dog was then placed into a thermally isolated box. A sensor attached to the hip recorded the motor activity.

The automatic equipment was then turned on to take over the control function. The clock mechanism recorded periods during which the animal was in a state of hypothermia at a temperature of 25-23°C. The rate of cooling of the dog's body on the average was 1°C every 15 min (4°C per hour). The assigned temperature of 25°C was reached by the animal after approximately 3 hours.

As the body temperature was automatically lowered, the respiration slowed down. The same data were obtained when the dog was cooled by manual control. The pulse rate decreased. Pulmonary ventilation also decreased (from 5-12 liters per minute in the initial state to 1.6-4.0 liters per minute at 22°C).

A recording of the electrical cardiac activity during the course of hypothermia made it possible to note the elongation of the cardiac cycle, from 0.32-0.36 sec at 38°C up to 0.60-0.74 sec at 22-24°C. The voltage decreased; sometimes the P notch was almost smoothed out, the T notch was extended and at a temperature of 24-23°C a negative wave appeared in it. With a further stay at lower temperature the T notch became negative. By the time the temperature of 25°C was reached, there was splitting of the QRS notch in the first derivation.

As the body temperature decreased, the arterial pressure dropped. At 22°C it was maintained at the level of 80 (maximum) and 30 mm Hg (minimum).

Thus, the duration (up to 18 hours) of the animal's stay in a state of hypothermia at 23-26°C did not change the values of the recorded indices, compared with the initial period of hypothermia (at a body temperature of 25-23°C). A change was observed only in the nature of the ECG--the occurrence of a negative notch T, which, in the opinion of a series of authors, indicates hypoxia of the myocardium.

During the period of hypothermia development, leukocytogenesis had a phase nature. Initially, when the body temperature was lowered by 2-8°C, the number of leukocytes in the peripheral blood increased from 13.1 ± 0.8 thousand cells per mm^3 to 18.8 ± 2.3 thousand cells one hour after the cooling was started. At the end of the cooling, i.e., at a body temperature of +25-22°C, their number decreased, frequently below the initial level (on the average down to 12.1 ± 1.0 thousand per mm^3 of blood). The erythrocyte content increased as hypothermia developed (on the average by 1 million cells at the end of hypothermia).

During the cooling process the hypothermia installation was stable in its operation. As we see from figure 2, the oscillation of the body temperature in the course of the experiment was approximately 4°C (22-26°C).

If frequency and depth of respiration decreased when the body temperature /596 was approximately 25-22°C, the automatic device turned on the oxygen supply. In isolated cases, the animal began to shiver at this point (there was a decrease in the depth of anesthesia). Then the nitrous oxide supply was turned on with oxygen, until deep anesthesia was reached.

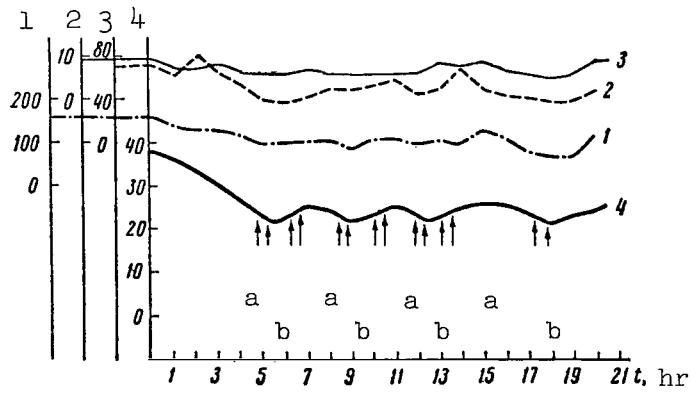


Figure 2. Variation in the arterial pressure (1) in mm Hg, respiration rate (2) and pulse rate (3) per minute, body temperature (4) in degrees C of dog Murat during hypothermia. a = cooling, b = heating.

After the animal had been at a body temperature of 20-25°C for a preassigned period of time, the clock mechanism turned off the cooling system and the heating of the animal was started. The heating was continued to a body temperature of 30°C, and the heating rate during the initial period from 22-23°C was 2°C per hour. Later (from 25°C) heating took place more rapidly (4°C per hour). Total heating time was approximately 2.5-3 hours.

During the heating period the arterial pressure increased and by the time the body had reached a temperature of 30°C it had reached its initial value. The pulse rate and the respiration rate also increased, but did not reach the initial value. The pulse rate remained slow, at about half its initial value. During heating the content of leukocytes in the peripheral blood increased at the proper statistical rate (from 12.1 ± 1.0 thousand to 16.3 ± 0.5 thousand cells per 1 mm^3 of blood). The quantity of erythrocytes remained high. The ECG during this period showed the shortened time cycle of cardiac activity. However, it still remained high compared to its initial value. The voltage of the notches was also below its initial value and the T notch remained negative.

When heating was completed, the automatic control was turned off, the operation wound at the hip was sutured and the supply of anesthesia was stopped. /597 As a rule, the dog awakened quite soon (during a period of 1-5 min). The dog opened its eyes and lifted its head. The respiration rate increased and shivering occurred frequently. Some of the animals started to walk.

When the supply of anesthesia was stopped at a lower body temperature (25-28°C), reaction to external stimulation was reduced and at a temperature of 22-25°C it was absent. On the second day the behavior of some of the animals was no different from their initial behavior. In the remainder there was some flabbiness and some timidness. Pulmonary ventilation was reduced. By the third day all dogs had usually returned to their initial state.

By observing dogs for a period of one year, and by observing one in particular which was subjected to cooling six times in the course of two years, no pronounced variation in the state of the animals was observed. This is rather well confirmed by the data of periodic weighing, thermometry and investigation of the peripheral blood picture, as shown in table 1.

As we can see from the table, the weight and temperature of the body, the leukocyte and the erythrocyte contents do not exhibit sharp deviations from normal oscillations which take place in healthy animals. The erythrocyte sedimentation reaction in all animals after hypothermia was retained at the level of initial values (3 mm per hour).

Thus, we can conclude from these observations that repeated hypothermia with a body temperature of 22-23°C and a duration of 3-5 hours does not produce persistent pathological variations in dogs during the posthypothermia period. The deviations determined in a series of cases (flabbiness, leukocytosis) were of short duration and as a rule disappeared 3 to 5 days after hypothermia.

TABLE I. VARIATION IN SOME OF THE INDICATORS OF THE STATE OF A DOG'S ORGANISM AT DIFFERENT PERIODS AFTER REPEATED HYPOTHERMIA.

Indicator	Time after experiment							
	Back-ground	5 days	15 days	30 days	3 months	6 months	9 months	1 year
Weight in kg	14.3	15.7	15.2	15.4	15.5	15.4	15.4	16.7
Temperature in °C	38.7 ±0.2	38.7 ±0.2	38.4 ±0.2	38.4 ±0.2	38.2 ±0.1	38.7 ±0.2	38.6 ±0.2	38.4 ±0.1
Leukocytes in thousands	13.1 ±0.8	12.3 ±0.5	13.4 ±0.3	11.0 ±1.8	12.7 ±2.6	11.2 ±1.5	10.0 ±1.0	10.1 ±1.3
Erythrocytes in millions	6.1 ±0.1	6.7 ±0.1	6.2 ±0.1	6.3 ±0.1	6.5 ±0.2	6.3 ±0.2	7.2 ±0.2	7.2 ±0.5

PROCESSING HUMAN EXCREMENT BY MEANS OF NATURALLY OCCURRING ALGAL AND BACTERIAL POPULATIONS

M. S. Rerberg, T. I. Vorob'yeva, R. I. Kuz'mina and I. M. Barkhatova

The basis for a possible method of maintaining life in a small closed /598 system may be found in the principle of an uninterrupted biological circulation which permits multiple utilization of a limited number of biogenic elements.

A closed cycle system consists of three basic elements: synthesis, consumption and reutilization, i.e., recirculation of the products of human vital activity.

We attempted to develop a method of biological treatment of human excreta and of reclaiming water from the latter. For this purpose we selected a green protococcoid alga, Chlorella vulgaris, in a naturally occurring association with bacteria.

The possibility of purifying waste liquids in biological ponds with the help of phytoplankton and bacterial flora has long been known. Water purified in biological ponds becomes so pure that it is released into open reservoirs.

Our problem was to obtain a greater degree of treating excreta and regenerating water with the greatest possible utilization of biogenic elements.

The experiments showed that a three-step culture process is required for extensive and complete treatment of excreta.

Prolonged multistage culture in flasks disclosed three zones of decomposition as judged by the presence of indicative protozoan organisms, the number of bacteria and nitrogen indexes (figs. 1 and 2).

According to the multistage method, culture of protococcoid algae /599 together with bacteria is accompanied by a fairly complete mineralization of the organic matter of excreta, by the creation of an algae biomass and by the production of secondary, humus-type organic matter.

The data pertaining to the quality of regenerated water derived from human excreta conform to most of the requirements of international standards (table 1). The above indexes were obtained according to content of organic matter, principally of the humus type, which is characteristic of treating organic compounds under aerobic conditions. The part of the humus which will not dissolve

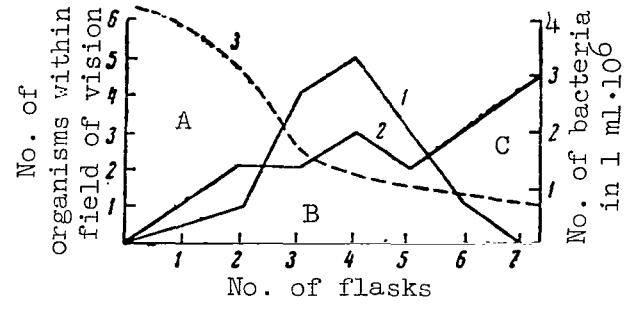


Figure 1. Distribution of stages of decomposition in flask. 1, Anisonema acinus; 2, Monas vulgaris; 3, bacteria. A, Poly-decomposition zone; B, α-mesodecomposition zone; C, β-mesode-composition zone.

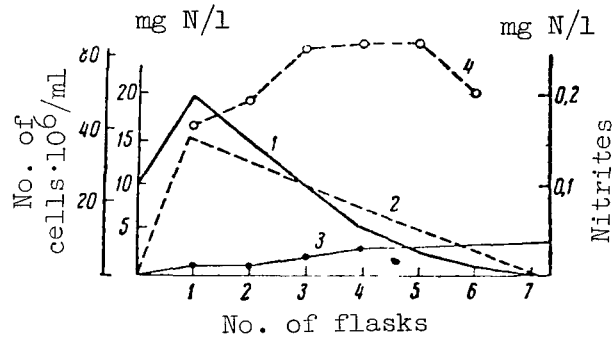


Figure 2. Distribution of nitrogen compounds in flask as result of work of algobacterial association. 1, NH_4; 2, NO_2; 3, NO_3; 4, density of algae cells per ml of medium.

in the water and which adds a turbid quality to it is easily precipitated by Fe-Al cations in an alkaline medium. /600

Upon precipitation of the humus, the water becomes transparent. Up to 30°C, its coloring falls within the platinic cobalt range; it smells weakly of rotten hay, but this odor quickly evaporates on settling. Purification of such water is easily achieved with physicochemical methods and does not result in any major loss of matter from the circulation.

Extensive cultivation of algae on human excreta was carried out experimentally for 10 months. No deterioration in the condition of the algobacterial system was noted at the end of the experiment.

A major defect of this method is the length of time required to treat a daily portion of excreta and to regenerate the water (1.5-2 months).

TABLE 1. RESULT OF BIOLOGICAL TREATMENT OF HUMAN EXCRETA BY ALGOBACTERIAL ASSOCIATION AS COMPARED WITH STANDARDS OF DIFFERENT COUNTRIES.

Country	Dry residue	Organic matter	H^o	Cl'	SO_4''	NO_3'	NO_2'	NH_4
	mg/l							
Belgium.	500	2-5	20	8	2-65	4	-	-
Switzerland.	500	2.5	-	20	-	20	0	0.02
France	500	2.0	-	40	-	-	0	0
USSR	500-600	2-3	19	20-30	80	30-40	trace	trace

Culture intensification of the algae made it possible to treat the medium in double concentration (dilution of 1:40 instead of 1:80) in only 3 days (illumination being provided 8 hours out of 24).

During this period, the medium underwent completely adequate treatment (table 2). The results following precipitation of solid secondary organic matter conformed to the requirements for water established for water supply sources ("GOST," 2761, 1957).

Chemical analyses made simultaneously for inflow and outflow, as well as chemical analyses of harvested algae cells, confirmed that most of the algae biomass consists of mineral carbon, supplied to the medium in the form of CO_2 (table 3). Note was made of the low ratio of C:N in Chlorella cells on emergence (C:N = 3 or 7), indicating a high protein content (averaging 53 to 72.8 percent).

Nitrogen was extracted rather completely from the medium. The algae biomass contained 82-85 percent of its nitrogen. On emergence the medium always contained small quantities of nitrate and ammonium nitrogen, amounting to 1.5-6 percent of the original amount. The remaining nitrogen in the medium consisted of ~ 10-12 percent (this nitrogen belonged to primary and mostly /601 to secondary organic matter, which was difficult to oxidize).

We observed nitrogen loss from cultures despite the presence of denitrifying microorganisms.

Phosphorus was usually absent in analyses of the medium. Furthermore we did not observe any precipitations of salts containing phosphorus on the walls of the culture vessels. Evidently all or almost all phosphorus is assimilated by the cells, as borne out by balance data. The great need of cells for phosphates (as compared with the experiment of culturing algae extensively) was due to the high degree of illumination and intensive operation of phosphorylation. The last was paralleled by a high degree of activity in protein biosynthesis. Magnesium comprised 0.42 percent of the dry weight of the algae,

TABLE 2. TREATMENT OF MEDIUM FROM HUMAN EXCRETA (1:40 DILUTION) BY MULTISTAGE METHOD IN LABORATORY CULTURES.

Medium from human excreta	NH_4	NO_3	NO_2	Albuminoid, N	Alkalinity, mg/eg	Hardness, mg/eg	Cl'	SO_4"	Mg	Ca	P	Fe_2O_3	Permanganate oxidizability
	mg/l						mg/l						
Before	8.5	4.5	0.04	15.2	2.6	2.5	180.00	137.7	11.2	25.90	23.5	0.1	100.8
After	0.34	1.07	0.001	-	2.5	1.1	176.40	97.5	8.16	9.5	0.00	0.05	35.6

the latter absorbed one-quarter of the total amount of magnesium initially present in the medium. Sulfur was used by the algae in insignificant amounts in relation to its content in the initial medium, consisting, according to our figures, of 0.72 percent of the dry weight of algae. The analyses did not disclose any chlorine in the algae. The basic contents of sulfur and chlorine remained in the medium on emergence in the form of sulfates and chlorides. Chlorella vulgaris, when cultured on human excreta, contained about 2 percent of calcium, consuming 2/5 of the total quantity of calcium in the medium.

Potassium was also only slightly removed from the medium, composing 0.7-1 percent of the dry weight of algae.

Having become convinced in principle of the possibility of treating human excreta with the aid of an algobacterial system and of the possibility of regenerating water, we attempted to close the system in regard to water for the purpose of determining what irreversible changes accumulate in such a /602
system and what factors are most destructive for a closed algobacterial system.

The experiment of culturing algae intensively on a urine-fecal substrate in a water-closed system occupied five months. It led to the following conclusions.

1. As might be expected, the quantitative imbalance between the mineral content of human excreta and the requirements of the organisms growing in the given medium and forming an ecological system (protococcoid alga Chlorella vulgaris + bacterial flora) precluded the assimilation of mineral salts. The latter first accumulated up to saturation and then precipitated out of the solution. This led to pH fluctuation of the medium in the culture flasks.

2. As a result of the five months' operation by the algobacterial system in a water-closed system, the medium accumulated many humus-type organic substances of primary and secondary origin, both soluble and insoluble. It was possible to precipitate the insoluble part of the humus, thereby facilitating the existence of both algae and bacteria. The soluble organic substances remaining in the liquid accumulated in large quantities. This interfered with the vital activity of Chlorella and associated bacteria and was finally responsible for destroying the system. Such destruction of the system could be prevented by removing the soluble organic compounds from time to time by physicochemical methods.

Preliminary experiments in treating human excreta under intensive conditions by round-the-clock illumination in an open circulation system with stabilized high density and an 8-10 dilution also demonstrated the pos- /603
sibility of biological utilization of excreta and the creation of large outputs of biomasses.

In a water-closed system, operating for 10 days under intensive conditions, even the first few days produced a good yield (28-47-55.5 g/liter). However, this was followed by the suppressive action of the medium as in the first closed experiments. It was later established in this connection that the suppressive actions of the medium were not due to the accumulation of sodium in it (up to

TABLE 3. BALANCE DATA FOR BASIC ELEMENTS (IN MG) INVOLVED IN PROCESS OF TREATING MEDIUM FROM HUMAN EXCRETA.

Element	Medium at outset (1:40 dilution)	Amount of element used by algae	Medium at end	Remainder
Carbon	118.3	1,640	36.9	13.6-fold deficit
Nitrogen	82.19	67.75	14.47	82.23
Phosphorus	23.5	22.76	0.0	22.76
Sulfur	18.86	5.86	13.0	18.86
Magnesium	11.2	3.04	8.16	11.2
Calcium	25.9	15.84	9.5	25.34

5 g/liter) or sulfur (up to 3 g/liter). They were primarily due to the large scale accumulation of soluble organic compounds. Vacuum distillation of the centrifuged products proved highly effective for removing the suppressive elements from the medium.

It is of interest to point out that special experiments under ordinary prolonged conditions demonstrated the stimulating action of NaCl on the algae cells when in a concentration in the medium of up to 2 g/liter. However, the algae are able to tolerate satisfactorily a concentration of NaCl of up to 4-5 g/liter.

For a more complete utilization of the elements in the medium and possible control over the treatment process, it is essential to standardize the human diet. This would correct the relationship of biogenic elements in the excreta by proper dilution or supplementation, where necessary, of missing salts in relation to the requirements of the system (algae + associated bacteria). If, for example, we are faced with the necessity of binding excessive sulfur and potassium, it becomes necessary to add nitrogen, phosphorus and magnesium to the original medium.

A preliminary study of microorganisms associated with algae in a medium containing human excreta, which grow in meat-peptone mediums and in a cellulose-containing medium showed that they belong to 6 families and 10 genera.

Among them there were many bacteria which cause ammonification by breaking down organic compounds containing nitrogen under aerobic conditions (microorganisms breaking down cellular tissue, humates, bacteria, soluble fats and potassium phosphates, which are difficult to dissolve). Flora of putrescent sporogenous microorganisms predominated under conditions which were unfavorable for the vital activity of algae by the suppressive influence of the medium and by the presence of many dead algae cells, detritus, soluble mineral salts and humates.

Our work shows the possibility of treating human excreta within a short space of time with the help of a naturally occurring algobacterial system. In

this way it was possible to obtain regenerated water which met the requirements expected of sources of water supply. However, further physicochemical conditioning is needed to render the water acceptable for drinking. Moreover, the possibility of microquantities of biologically active substances from the excreta remaining in the water has not been studied. /604

The advantage of a biological cycle utilizing an algobacterial association lies in combining four functions in a single process: synthesis of primary biomass, utilization of the bulk of excreta, primary purification of water and regeneration of oxygen.

PHYSIOLOGICAL INVESTIGATIONS OF SPEECH PROCESSES IN CONNECTION WITH THE PROBLEMS OF ESTABLISHING MAN-MACHINE COMMUNICATION BASED ON SPEECH

V. A. Kozhevnikov and L. A. Chistovich

The problem of direct communication of man with computers and /605
control systems becomes particularly significant under conditions of manned spaceflights (refs. 7, 13 and 14). The lever and button control frequently becomes unsatisfactory because, for example, of the limitations imposed on the movement of a cosmonaut during powered flight, when coordination can be lost under conditions of weightlessness, etc. In addition, a channel with a button input has a low carrying capacity, which is further limited by the preoccupation of the cosmonaut (ref. 6) with a great deal of information and the possibility of pilot errors under extreme conditions when very rapid decisions are required. Therefore, the problem of using a direct communication between man and machine by means of speech, which has great potentialities and which is not disrupted under conditions of weightlessness (ref. 4), is quite urgent.

The first stage in the development of a speech communication system with a machine is the development of an automatic "machine" system for the recognition of speech signals. The optimum version of the automatic recognition is considered to be the phonemic recognition: the transformation of the continuous speech fed to the recognition device into a sequence of code combinations, each of which corresponds to a phoneme--the smallest unit of speech that distinguishes one utterance from another when used to describe a language.

The common approach to the solution of this problem, which is /606
characteristic of most work carried out in the last 10-15 years, involves the following. First of all, certain procedures are used to break up the speech automatically into a sequence of sections corresponding to speech sounds. Then, by comparing each of the sections with a collection of standards describing the phonemes, it is necessary to find the particular phoneme standard which is most closely approached by the given section from the standpoint of its spectral and other characteristics (refs. 15 and 26). However, this approach, which is very attractive because of its apparent simplicity, has not achieved substantial success to date. Unfortunately there is also a series of theoretical considerations based on data obtained from phonetic investigations (ref. 8), which indicate that this approach is not very promising.

In the last two to three years a new approach to this problem has evolved and has been formulated most clearly by Stevens (ref. 29). The basic idea of this approach is that the first stage of recognition is the determination of

articulation parameters, used to produce an acoustic signal from the properties of this signal. The second stage of recognition is concerned with the transformation of data on articulation into discrete symbols capable of distinguishing meanings (sequence of phonemes, words).

This approach is supported first of all by the unalterable fact that the discrete units which distinguish one utterance from another can exist in the speech process only at the level of the control programs for the articulation organs. Due to the inertia of the articulation organs and due to insufficient synchronization in their activity, the discrete features do not exist at the level of acoustic speech representation, which, in itself, represents a continuous process. However, since the properties of an acoustic signal are determined completely by the characteristics of articulation, the variation in the signal properties can, in principle, be used to determine the articulation movements and from them the element which distinguishes one meaning from another--the collection of commands supplied to the articulation organs.

Another important argument in favor of this approach to the automatic recognition of speech is the established fact that the first stage of speech recognition by man is also the determination of articulation signs for the received signal (refs. 16, 17 and 24).

However, this new approach to the automatic recognition of speech is not sufficiently well developed at the present time: neither with respect to the determination of articulation parameters from the acoustic properties of the speech signal, nor with respect to the transition from articulation parameters to solutions concerning the discrete elements distinguishing one utterance from another. In the work carried out at the Massachusetts Institute of Technology (U.S.A.) information on the parameters which describe articulation are obtained by means of a method called "analysis through synthesis" (refs. 19 and 29). /607

The basis of the method is the comparison of each of the successive speech signal spectra with spectra synthesized by a machine in accordance with rules based on the acoustic theory of speech production (ref. 22). As a result of this comparison a synthesized spectrum is developed, differing as little as possible from the output spectrum, and the meaning of the signal at any given instant of time is described by the collection of parameters characterizing this synthesized spectrum. Thus, the entire investigated speech signal (sound, syllable, word) is represented by a limited number of functions, each of which reflects the behavior of a specific parameter with time.

Judging from the published data, this method is sufficiently accurate; however, the procedure itself of finding parameters for which the synthesized spectrum corresponds to the input spectrum in the best fashion requires a considerable expenditure of time and the use of rather bulky equipment (if the entire collection of spectra being compared is assigned simultaneously).

It is obvious that a detailed description of the behavior of various parameters in time is quite important in the investigation; however, we can also assume that for recognition purposes it is not particularly important to know the detailed state of each parameter at each instant of time. However, it is

important to diagnose the nature of the variation of a given parameter at the instant of time when this variation takes place. We can assume that the solution of this problem can be achieved by simpler methods of detecting such variations in the system which follow the variations in the important articulation parameters in a regular fashion.

The searches for useful acoustic indicators of the speech signal may proceed in at least two directions. One of these is to make a detailed investigation of the acoustic signs and recognition strategy used by man who determines from the speech signal those articulation motions performed when it is pronounced. However, if these investigations are to have a quantitative nature, it is necessary to have, in conjunction with the acoustic signal, data on its articulation parameters and to have the means of isolating such sections of the acoustic signal corresponding to definite articulation states.

Another direction consists of using statistical processing (due to its bulkiness, it must be automated) to isolate characteristic signs corresponding to definite articulation states and to transitions from one state to another. To carry out this problem we must also carry out a parallel recording of articulation parameters and of the acoustic signal.

The next problem, which is now no less complicated, consists of estab- /608
lishing procedures which can be used to go from data on articulation speech parameters to decisions concerning the discrete elements which separate intelligence. As we have stated, the elements which distinguish meaning during the process of speech formation can exist in their pure form only at the level of the programs for the control of articulation organs. Therefore, in order to find the optimum methods of isolating them based on the articulation parameters, it is necessary to carry out fundamental investigations to establish the nature of articulation programs, their principles of organization and the mechanisms for the realization of these programs.

To date, the problems remain completely unstudied. The necessary prerequisite for beginning investigations in this direction is the use of such methods which would make it possible to record the articulation dynamics of speech in sufficiently comprehensive form. Unfortunately, it must be realized that the methods of recording articulation indicators in the dynamics of normal speech are not sufficiently well developed, although even a brief acquaintance with the experimental phonetics, which developed into an independent discipline at the end of the last century, shows that all its substantial developments were determined by the existing methods of recording articulation then at the disposal of the investigators.

The method which has been widely used by the physiologists and which has survived to our day is the pneumatic technique of recording. This uses little drums with rubber membranes, air tubes and a kymograph with sooty paper (refs. 3, 18 and 27). Kymograms can be used to make a satisfactory recording of the many peculiarities of the respiratory motion. This is of importance for understanding the mechanisms generating speech sounds and, to some extent, the air currents from the stomatic and nasal cavities associated with phonation,

intra-oral pressure, etc. This technique reached its highest point of perfection and complexity in the investigation by Stetson (ref. 28).

An important method, widely used in phonetics, is the method of palatography--a method of obtaining prints of the contact region between the tongue and the hard palate when various sounds are pronounced (refs. 3 and 23).

By using methods of this type, phoneticians have developed a rather complete description of the configuration of the speech tract and the peculiarities of air flow which take place when various speech sounds are pronounced.

However, due to the limitations of kymographic recording, the description of articulation time dynamics and the relationship between articulation and acoustic pictures of speech are less amenable to description. Approximately in the mid-30's the basic attention of phoneticians was focused on the electroacoustic and radio electronic methods, which made it possible to carry out /609 very fine and exhaustive analyses of the physical properties of speech sounds (refs. 2 and 22).

However, the development of methods for the direct recording of articulation phenomena was substantially slowed down. It is true that a series of important data were obtained on vowels, primarily for the investigation of phonation, by means of various approaches using the X-ray technique (refs. 1, 12 and 30). Recently many investigators have been attracted by the electromyography of muscles participating in the speech process (refs. 10, 21 and 25). However, methods which would permit a sufficiently complete recording of the articulation picture and the comparison of its peculiarities with the acoustic flow of speech, as far as we can judge from the published literature, have not been applied to date in a systematic manner by any single investigator.

As a result the first problem of investigations in this direction is a need for developing methods for comprehensive recording of such speech tract indicators which reflect with sufficient completeness and in a continuous fashion the articulation dynamics in parallel with the acoustic picture of speech. It has also become important to obtain experimental data with sufficient accuracy and in large volume.

The basic problem in the development of experimental methods is the design of a system which will permit the study of articulation phenomena in the dynamics of normal conversational speech, and which will provide a sufficiently accurate correlation of the acoustic picture with definite articulation facts. After the various principles of the electrical measurement of nonelectrical quantities were reviewed and applied to the fabrication and testing of sensors for articulation parameters, the methods described earlier were selected (refs. 27 and 28).

The signals generated by the collection of sensors (fig. 1), in spite of a relative limitation in the number of measured parameters, reflect to a certain degree all three basic sides associated with the operation of speech production organs (the creation of the air stream, required for the excitation of acoustic oscillations; articulation dynamics, which provides for the modulation of this

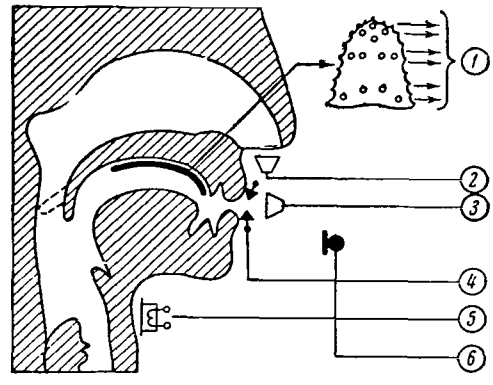


Figure 1. Arrangement of basic sensors to measure articulation parameters of speech. 1, "artificial palate" sensor which registers contact of tongue with hard palate; 2, sensor which registers flow of air through nasal cavities; 3, sensor which records flow of air through mouth opening; 4, sensor which records joining of lips; 5, throat microphone which records origin of vocal chord oscillations; 6, microphone which records acoustic picture of speech.

flow and which is the basis for all complex sequences of sounds forming speech and finally the occurrence of acoustic oscillations composing the speech signal in the proper sense of the word).

We present several examples. If signals are supplied by two sensors recording the air flow (from any of the sensors or from both of them at the same time), this shows that the air is moving in the speech tract. The flow /610 of air through the mouth only shows that the palate curtain is raised, thereby cutting off the air paths through the nasal cavities; the flow which is recorded only in the nasal opening, on the other hand, is peculiar only to the nasalized sounds. Significant data on articulation are carried by signals which indicate that there is a closure of the lips and that the tongue touches the palate. The time dynamics of these contacts, together with the possibility of establishing the point of contact between the tongue and the palate, characterized to a significant degree the special features associated with the pronunciation of a series of consonant sounds.

The importance of information obtained by means of the "artificial palate" sensor has made it necessary specifically to examine the accuracy in localizing the contact point, the reliability of recording and the problems of selecting the voltages for feeding the sensors. The results of this investigation are contained in the article by Yu. I. Kuz'min contained in the present volume.

We should point out that the discovery of many aspects of speech time dynamics is aided even by a radically simplified reflection of articulation organ activity, observed by means of the sensors, specifically the recording of the mere facts of presence or absence of respective articulation phenomena. The representation of the signal in this case may be simplified to the /611 "binary form," while the system of the speech producing tract is analyzed as a sequence of discrete actions, each of which may have only two positions, "on" or "off."

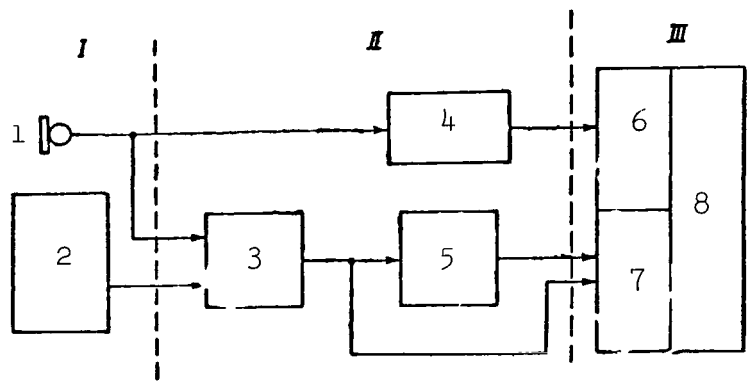
Such a system cannot achieve a complete simulation of the speech production tract during the generation of vowels, because in the latter case the key factors are associated with the stress of the vocal chords and the value of the pressure below the slit, the volume and other gemoetric peculiarities of the resonant cavities, and also a series of other phenomena whose amplitude may change continuously (refs. 20 and 22). However, the measured parameters reflect many of the key features associated with the articulation of consonants: the nature of lip contact of prepalatal, midpalatal and postpalatal contacts, nasalization, clearness or dullness and several other signs of individual consonants (refs. 11, 16 and 17).

The volume of information obtained during the investigation of the continuous flow of conversation speech by means of the system of these sensors turns out to be quite substantial. For this reason it becomes particularly important to consider rational methods of recording the respective phenomena and the maximum automation of measurements.

Many aspects of the articulation dynamics can be studied with success by recording all observed phenomena with a multichannel pen recorder. However, due to the inertia of the pen, such a system is somewhat limited in the study of more rapid processes.

The possibilities of the investigations are substantially expanded when procedures are used involving the automatic processing of data by means of corresponding electronic devices. The signals in the system developed by V. S. Shuplyakov and described in the present volume are subject to a two-stage processing. First, the initial voltages generated by the sensors are automatically converted into highly simplified signals of "binary form" showing only presence or absence of the respective articulation parameter. During the second stage the information is processed in a logic fashion by means of electronic circuits which contain the rules for determining the group of signs characterizing the unknown phenomena.

The pulse signals obtained during the first stage characterize the duration of initial articulation parameters, while the secondary signals shaped by means of logic circuits characterize the duration of respective, more complex, phonetic groups. All signals may be used to control an electronic gate (refs. 8 and 30) which isolates and records sections of the speech acoustics on a magnetic tape recorder, corresponding exactly to specific articulation phenomena (fig. 2). /612



1. Microphone
2. Sensors of articulation parameters
3. Preliminary signal processing stages
4. Electronic gate
5. Logic units for processing signals
6. Segments of acoustic signal
7. Articulation parameters
8. Synchronous recording and automatic measurement

Figure 2. System for processing information in experiments on speech process. I, generation of signals by sensor; II, preliminary and logic processing of signal; III, recording and automatic measurement of specific properties of initial process.

The preliminary simplification of initial signals and the use of electronic logic circuits also simplifies the automatic measurement of time variations in the articulation dynamics of speech. The measurements in this case consist of establishing the time intervals between the leading edges of respective impulses. In this case it is easy to use electronic techniques to express the results directly in digital form, and also to automate certain statistical evaluations of the material obtained in a rather large volume.

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SOME METHODS FOR RECORDING AND PROCESSING INFORMATION IN INVESTIGATING THE ARTICULATORY INDICES OF SPEECH

V. S. Shuplyakov

To investigate speech under dynamic conditions we developed and /614
fabricated a collection of sensors (refs. 2 and 3), with which to record a series of functions performed by the sound-producing apparatus with a sufficient degree of accuracy.

All signals generated by the sensors were electric. The basic device which recorded them was a 16 channel pen recorder. Since the investigation was concerned with the establishment of accurate amplitude-time relationships between signals arriving from different elements of the speech-producing tract, and the reception of various statistical data, it was necessary to conduct a meticulous graphical processing of the recordings. Not only is the processing very difficult and inaccurate but, in a series of cases, becomes completely infeasible.

For example, in our experiments we compared a combination of articulation parameters with the acoustic picture of the speech pronounced during this time. Due to the insufficient bandpass of the recorder (from 0-100 cps), the latter cannot be recorded on it directly. In addition the result of the graphic processing of data always turns out to be substantially displaced in time with respect to the initial signals, which also impairs the experimental conditions.

Our method permitted us to carry out various time measurements automatically and to process the initial data logically, thereby obtaining complex signals which reflect a whole series of speech properties, important from the standpoint of phonetics. This made it possible for us to study the segmentation of continuously flowing speech sounds, based on different combinations of articulation parameters. /615

To carry out the logic operations we reduced the diverse signals from various sensors to a single form. Since the basic purpose of the investigation was to consider the amplitude-time characteristics of the articulation process, we carried out the required unification of the signals without reducing substantially the information of interest to us. The transformation of the signals into rectangular impulses by means of amplitude selectors and shaping circuits basically satisfies this requirement. Signals of rectangular form and of the same amplitude also have the advantage that they can be fed directly to the electronic circuits which perform various logic functions.

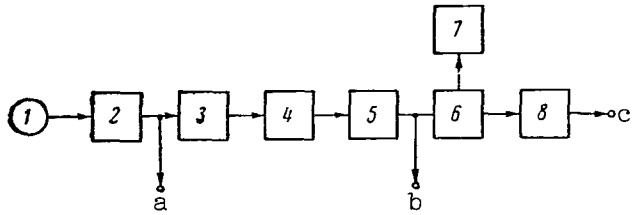

Figure 1. Functional diagram of channel for processing signals of articulation parameters. 1, sensors; 2, amplifier; 3, phase inverter; 4, detector; 5, integrating network; 6, amplitude selector; 7, source of stabilized reference voltage; 8, shaping circuit; a, output to control level of selection; b, output forming envelope on pen recorder and controlling level of selection; c, output of shaped signal to logic circuits which process and control selection.

The equipment we developed consists of a 10 channel device, such that each channel transforms the signals from the sensors into rectangular impulses. It also consists of logic circuits--decoders and an acoustic separator, which controls the articulation parameters and recording equipment. The functional diagram (fig. 1) of one of the channels consists of the following stages: amplifier with a phase inverter, detector, integrating networks, amplitude selector and shaping circuits.

Signals from the sensors which are biased with audio currents are amplified by a conventional two-stage RC-amplifier with a response of 20 cps-20 kcps and an amplification factor of approximately 300. To improve conditions for the subsequent detection, the output of the amplifier has a self- /616
balancing phase inverter whose circuit uses cathode coupling. The conventional full-wave detector utilizes semiconductor diodes. To obtain the envelope, a smoothing RC-network is used with a time constant of the order of 5 msec. All signals of the envelopes may be recorded by means of a multichannel pen recorder and are also fed into a system for automatic processing of data.

An important procedure in the automation of measurements turned out to be the selection of useful signals showing that a respective articulation parameter is or is not present. When the signal exceeded a certain assigned amplitude, this served as a criterion for the selection. The amplitude selection also becomes quite difficult due to the presence of various interferences: the instability in the sensor signals produced by the carrier frequency, and others. The amplitude selector consists of a difference amplifier. One of its outputs is fed with the signal voltage from the integrating network, while the other is fed with a comparison voltage. The magnitude of the comparison voltage determines the level of selection, and if necessary may be varied. In practice this voltage serves as a reference level such that any excess is transmitted to the subsequent shaping circuit. The final shaping of the signal into a rectangular impulse takes place in the Schmidt trigger.

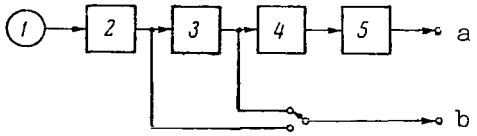

Figure 2. Block diagram showing selection level control. 1, sensor; 2, amplifier with phase inverter; 3, detector with integrating network; 4, amplitude selector with shaping circuit; 5, controlled brightness biasing generator; a, output to brightness modulator of oscilloscope; b, output to vertical amplifier of oscilloscope.

The setting of the selection level plays an important role in the correct determination of the fact that parameters are present. In connection with this, provision is made for visual control of this level during the course of the experiment, which is achieved by means of circuits shown in figure 2a. The shaped rectangular signal at the output of the channel whose selection level must be set is fed to the grid of a multivibrator, which is blocked when there is no signal. As a result the latter is excited and generates oscillations with a frequency of approximately 50 kcps. The impulses from the output of /617 the multivibrator are fed to the circuit which modulates the brightness of the cathode ray tube beam. Simultaneously, the output of the vertical amplifier is fed with the envelope of the selected signal. The screen of the oscilloscope shows the entire envelope, and the region which is taken as the signal is illuminated by the impulses from the multivibrator. The outputs of the envelopes of all channels are fed to the oscilloscope through a common switch, so that the level of selection in all channels can be rapidly controlled and set.

In view of this method for the shaping signals, all measured parameters are represented in binary form and can be processed by binary logic. The phenomenon of articulation, which is of interest to us, may in this case be represented in the form of tables or rules describing certain definite relationships between parameters.

For example, after the graphic processing of data, recorded by several channels of the pen recorder (ref. 3), we determined the following relatively simple logic relationships between the measured articulation parameters, which determined the presence of clearness in certain pairs of labial and nonpalatalized constants (p - b, t - d, s - z and the corresponding soft sounds $p_з$ - $b_з$, $t_з$ - $d_з$, $s_з$ - $z_з$).[1] The existence of clearness corresponds to the coincidence of the following signals: a positive signal from the throat microphone channel, which is delayed by a time interval of the order of 30 msec, and the signal which shows that the prepalatal contact has ended (the trailing edge of the signal from contact No. 3[2] of the "artificial palate" sensor), or the signal

[1]International Phonetic Alphabet (I. P. A.) symbols.
[2]The numbering of the contacts follows the method of Yu. I. Kuz'min (ref. 3).

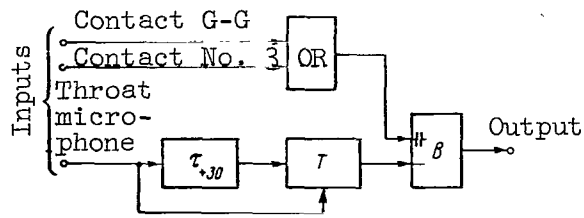

Figure 3. Block diagram of automatic device to detect presence of clear lip and prepalatal consonants. B, rectifier; τ_{+30}, circuit which delays impulse by 30 msec and which is fired by positive impulses; T, trigger fired by negative impulses; OR, circuit to perform logic operation OR; contact G-G, output signal of sensor channel for juncture of lips; contact No. 3, the output signal of channel for contact No. 3 of artificial palate sensor; throat microphone, output signal from throat microphone.

which shows that the juncture of lips has ended (the trailing edge of the sensor signal for lip contact).

The structural diagram of the device which performs these simple operations is shown in figure 3. The signal at the output of this system appears every time when the amplitude-time combination of input parameters follows the rule stated above.

In specific experiments it is necessary to have a circuit which performs other logic operations. It may also become necessary to have complicated systems of such circuits operating in parallel or in series. Such logic circuits, which represent simple decoders, may be used to isolate automatically signals with preestablished parameters. In order to change the logic problems rapidly, we fabricated different potential tube logic elements in the form of plug-in units with identical contact arrangements and with identical power supply requirements. With this unification of the logic elements the replacement of a logic problem is reduced to the switching of certain connecting /618 wires and may be accomplished quite rapidly. If the signals fed from such a logic device are to be controlled by means of an electronic gate or by means of an acoustic separator (ref. 1), the separator will transmit acoustic signals during the time instants when there is a strict correspondence to definite logic combinations of articulation parameters. Segments of the acoustic signals selected by means of such a gate are fed to the recording equipment, where they are recorded with all details, and, if necessary, can be measured automatically or heard.

In addition to the 16 channel pen recorder the type N-102 galvanometer oscillograph was used as well as a type MEZ-28 magnetic tape recorder.

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THE ROLE OF VISIBLE ARTICULATION IN SPEECH RECOGNITION

V. V. Alyakrinskiy

It is known that it is easier to recognize speech when the face of /619
the speaker is visible. It has been shown that the observation of a speaker's articulation substantially increased the intelligibility of oral speech in the presence of background noise (ref. 14). Thus the visible manifestations of articulation may serve as a valuable supplement in the acoustic recognition of distorted speech signals. This induced us to investigate the limits for visual recognition of speech by people who lost their hearing completely and cannot recognize speech sounds acoustically.

The investigations of speech recognition by deaf people from the visible manifestations of articulation were conducted for two purposes: first, to determine the information of speech signals which can be obtained by observing the speaker's face, and second, to investigate the special features associated with the reception and processing of speech information when a person cannot obtain complete information on each phoneme and must base his decisions only on the visual articulation which is characteristic of several phonemes at the same time.

Experience with people who became deaf after they had learned how to speak shows that by special training some of the people can effectively recognize speech from visual articulation. For this reason I. A. Vasil'yev (ref. 3) assumed that a definite visible articulation position corresponds to each speech sound. However, the articulation of the same sound is known to vary with the phoneme that precedes or follows that sound. Naturally this makes the visible recognition of speech more difficult. In addition, the visual reception of speech sound articulations is influenced by the special features of the speaker's speech apparatus and of his pronunciation.

In the works devoted to the teaching of "lipreading" (refs. 3, 6 and /620
7) experimental investigations of the visual recognition of speech were not carried out. These problems were considered by F. F. Rau and B. I. Khitrik. However, their works were not published and only some of the results are presented in the monograph by V. I. Bel'tyukov (ref. 2). In evaluating visual recognition, the authors were concerned only with intelligibility, and from the data presented it is not possible to establish how clearly the individual groups of consonants are isolated or how the consonants within these groups are recognized.

To clarify these problems, in addition to determining intelligibility, we computed the amount of received information (ref. 12) and analyzed the distribution of errors in the visual recognition of speech.

The subjects were six students from senior classes of two schools for persons suffering from late deafness. Audiometric tests showed that all subjects were completely deaf. All subjects lost their hearing when they were between the ages of 5 and 9, i.e., when they already had command of speech.

The experimental material consisted of 250 meaningless syllables prepared at the phonetics laboratory of the Leningrad University, taking into account all the possible sound combinations in the Russian language (ref. 5). In addition, in order to clarify the difference in the recognition of accented and unaccented vowels, 200 words and sound combinations consisting of two open syllables were pronounced. In half the sound combinations, the accent was on the first syllable, while in the other half it was on the second syllable. All information was pronounced by a single speaker. The test subjects carefully followed the articulation movements and immediately wrote down what they thought had been pronounced.

The written answers of the test subjects were used to prepare matrices which reflected the nature of the visual recognition of vowels and consonants. The data of the matrices was used to determine the intelligibility, the amount of transmitted and received information and the analysis of error distribution. During the first experiment with three subjects the repetition of syllable pronunciation during the reading was permitted. During the second experiment with the same group carried out after one month and also during experiments with other groups, the syllables and words were pronounced only once.

During the transmission of meaningless syllables the total intelligibility of vowels was 78 percent. When words were transmitted, the total intelligibility of accented vowels was 75 percent and was 44 percent for the unaccented vowels.

The intelligibility of individual vowels during the transmission of meaningless symbols differed (fig. 1, I). The labialized vowels o and u were best recognized.[1] The test subject identified them in 98 and 92 percent of the cases. The errors in the recognition of a and ɛ occurred because these sounds were mistaken for each other. /621

The vowel sound ɨ was the one most difficult to recognize. Its intelligibility did not exceed 38 percent and in most cases it was recorded as the vowel i. The same results were obtained in establishing the intelligibility of accented vowels in words (fig. 1, II). The intelligibility of the accented ɨ was also the lowest: in 47 percent of the cases it was recorded as the vowel i. The possible explanation for this phenomenon will be presented below when we consider the effect of the linguistic probabilities of the Russian language on the visual recognition of speech sounds.

[1]International Phonetic Alphabet (I.P.A.) symbols: а = a, и = i, ы = ɨ, е = ɛ, о = o, у = u.--Tr.

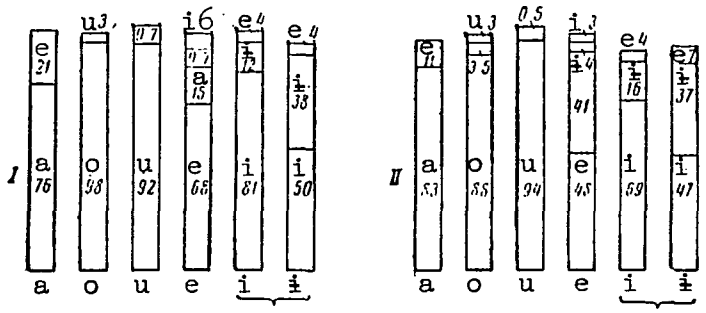


Figure 1. Intelligibility of vowels I in syllables (in percent). Intelligibility of accented II vowels in two syllable words and in combinations (in percent).

TABLE 1. AMOUNT OF INFORMATION RECEIVED DURING VISUAL RECOGNITION OF VOWELS IN SYLLABLES (IN BINARY UNITS).

Test subjects	With 6 vowels	With 5 vowels (ɨ and i considered one)
G., R., Kh. Recording 1 . .	1.67	1.64
G., R., Kh. Recording 2 . .	1.53	1.51
A., S., E. Recording 1 . .	1.82	1.81
Average	1.67	1.65

Frequent incorrect recording of the vowel i instead of ɨ posed a question as to whether the test subjects can distinguish these vowels visually. We computed the amount of received information twice. First we assumed that all six vowels were transmitted while in the second case it was assumed that only five vowels were, since i and ɨ were considered one. If ɨ and i are distinguished by the subjects, the information received first should be greater than the amount received next. Table 1 shows that although the amount varies (different test subjects: in the first experiment repetition of individual syllables was permitted; in the second, syllables were pronounced only once), the amount of information received in both was almost the same. Thus we assume that ɨ /622 and i are of one articulation group and that the subjects do not distinguish between them during "face reading." In the transmission of syllables and words there were cases when individual vowels were not recognized, i.e., neither received nor replaced with other vowels. The total of unrecognized vowels in the syllables was 1.8 percent. The accented vowels in words were not received during 4.8 percent of the time, while the unaccented vowels were not received during 24.7 percent. Data for vowels are in table 2, which shows that when syllables and accented and nonaccented vowels are transmitted, ɨ is missed most

TABLE 2. NUMBER OF VOWELS IN WORDS AND SYLLABLES WHICH WERE NOT RECEIVED (IN PERCENT).*

Vowels	a	o	u	ɛ	i	ɨ
In syllables	1.6	0.5	0.4	2.4	2.7	5.8
Accented vowels in words	3.9	4.8	0.8	3.1	10.2	10.0
Unaccented vowels in words . . .	16.4	23.5	20.2	32.6	41.0	50.0

*Data obtained from six subjects.

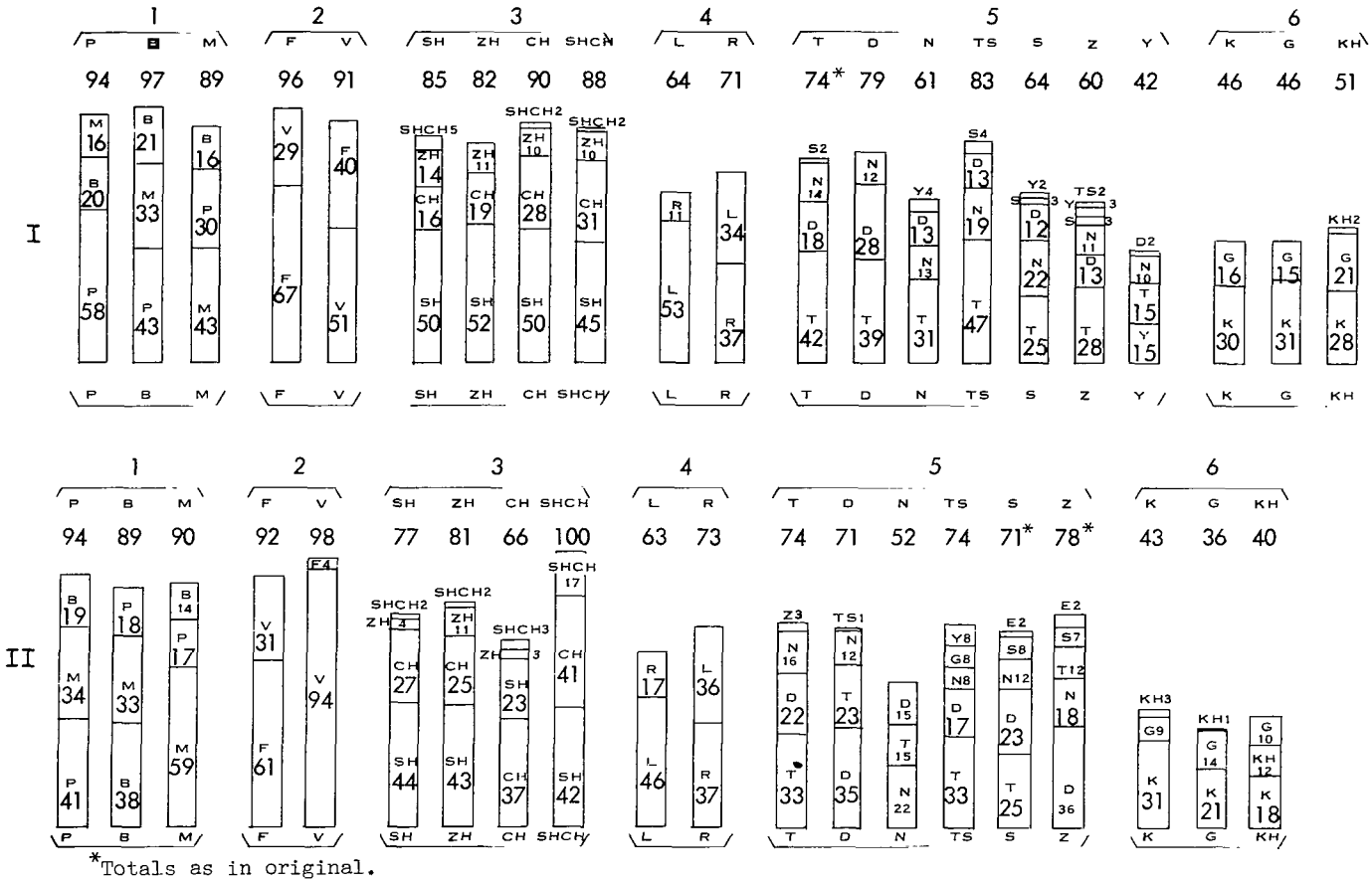
frequently and then the vowel i. The remaining vowels were undetected in a large number of cases when they were unaccented vowels in words.

Visual recognition of consonants when meaningless syllables were /623 transmitted was much worse than the recognition of vowels. The total intelligibility of consonants did not exceed 30 percent. In considering the intelligibility of individual consonants and the nature of error distribution (fig. 2, I), the group recognition of consonants was clearly established. In this case, when the test subjects make an error in the determination of some consonant, they record another consonant from the same articulation group. Six such groups were detected: (1) labial stops (p, b, m); (2) labial fricatives (f, v); (3) front (ʃ (sh), ʒ (zh), ʧ (ch), ʃʧ (shch)); (4) front liquids (l, r); (5) front stops (t, d, n, ts), fricatives (s, z) and mediolingual sonant (ɨ), and (6) postlingual consonants (k, g, x).

The special feature of visual recognition of consonants is that the test subjects who determined the consonants of some group usually selected the voiceless consonant of this group. For example, in the group p, b, m they recorded p most frequently, while in the group ʃ, ʒ, ʧ, ʃʧ the subjects preferred to cite the consonant ʃ, while in group t, d, ʧ, ts, s, z, ɨ the consonant t and in the group k, g, x the voiceless consonant k. /624

When the intelligibility of the group was high, the intelligibility of individual consonants was very low. Thus, the group intelligibility of the consonants m, ʒ, ʧ, ʃʧ was 82-90 percent, while the intelligibility of the consonant ʒ was 11 percent and of the consonant ʃʧ was only 2 percent.

Furthermore, the intelligibility of the group t, d, n, ts, s, z, ɨ varied from 42-83 percent, while the intelligibility of the consonant n was 13 percent, of the consonant s it was 3 percent and of the consonant z it was 1 percent. The consonant ts was not correctly identified at any time. The intelligibility of the group k, g, x was 46-51 percent while the intelligibility of the consonant x was only 2 percent. Similar results were obtained when consonants were transmitted in words (fig. 2, II).



*Totals as in original.

Figure 2. Group intelligibility of I consonants in syllables (in percent). Group intelligibility of II consonants in words and two-syllable combinations (in percent).

The decrease in the group intelligibility in the 4th-6th groups of consonants, compared with the first three groups (fig. 2), was partially due to the fact that in these groups the number of consonants not received at all increased (table 3).

As we see from table 3, the visual recognition of postpalatal consonants (k, g, x)[1] was most difficult and was accompanied by the largest percentage of unrecognized consonants. This becomes understandable if we remember that during pronunciation of postpalatal consonants the visible manifestations of articulation are at a minimum.

[1]I.P.A. x = kh, B.G.N. transliteration.--Tr.

TABLE 3. AVERAGE PERCENTAGE OF CONSONANTS IN EACH ARTICULATION GROUP WHICH WERE NOT RECOGNIZED.

	Groups of consonants					
	p,b,m	f,v	ʃ,ʒ, ʧ,ʤ	l,r	t,d,n,ts, s,z,i̯	k,g,x
In syllables . .	4.2	3.8	3.4	16.4	17.4	35.0
In words	5.7	4.5	8.0	14.5	16.5	25.0

TABLE 4. QUANTITY OF RECEIVED INFORMATION DURING THE RECOGNITION OF CONSONANTS IN THE COURSE OF VISUAL ARTICULATION (IN BINARY UNITS).

Test subjects	Consonants not combined into groups	Consonants combined into 6 groups
G., R., Kh. Recording 1	2.14	1.67
G., R., Kh. Recording 2	1.77	1.67
A., S., E. Recording 1	1.97	1.79
Average	1.96	1.71

The substantial difference between the group intelligibility and the intelligibility of individual consonants led us to propose that the test subjects recognize only groups of consonants; however, since they were required to write down a definite consonant, they selected one at random from those consonants possible in this group. To verify this proposition, we double-checked the quantity of received information: in the first case it was assumed that each consonant was transmitted separately, while in the second case it was assumed that only six articulation groups combining all consonants were transmitted (table 4).

Although by combining consonants into groups we decreased by two the amount of transmitted information, the quantity of received information decreased on the average from 1.96 to 1.71 binary units, i.e., by 0.25 bits during the transmission of syllables. Similar calculations of the amount of information received from transmitted words showed that the combination of consonants into six articulation groups on the average reduced the amount of information received by only 0.03 bits. /625

If the combination of all consonants into six groups has almost no effect on the received information, it follows that the differentiation between consonants in each group is very small. The question therefore arises whether the

TABLE 5. AMOUNT OF RECEIVED INFORMATION INSIDE EACH GROUP OF CONSONANTS (IN PERCENT).

Test subjects	p,b,m	f,v	ʃ,ʒ,tʃ,ʧ	l,r	t,d,n,ts, s,z,ɨ	k,g,x
G., R., Kh. Recording 1.	5	4	7	14	3	3
G., R., Kh. Recording 2.	4	7	3	11	6	4
A., S., E. Recording 1.	6	3	5	6	3	5

low recognition of consonants in all groups is the same. The calculations of the amount of received information inside each group of consonants is shown in table 5.

It was clear that the magnitude of consonant differentiation in all groups was low. The received information in most cases was 3-7 percent of the transmitted information. Only in the group l,r did the percent of received information reach 11-14. It is possible that this is associated with the fact /626 that during "face reading" the difference in the articulation positions of these two consonants is more noticeable than the difference for consonants of other groups.

These data confirmed our proposition that the test subjects, during the visual recognition of visible articulation of consonants, in fact distinguish only groups, while the individual consonants inside these groups are practically not differentiated.

Thus, during "face reading" only 11 articulations are recognized visually: 5 vowel articulations and 6 consonant articulations.

We may now ask how the test subjects proceed from 11 articulation groups to 42 phonemes of coherent speech.

The subjects must make decisions under the following conditions: they can see, for example, whether the speaker has closed or opened his lips. The most they can learn from this is that the speaker has pronounced the consonants p, b, m, and that they must write down one of these three possible sounds.

It would be natural to assume that in selecting one of these sounds a person utilizes the linguistic probabilities, i.e., the information which he has concerning the relative frequency of occurrence of these sounds. Analysis of the errors has shown that such information is indeed utilized.

As we have shown (by determining the intelligibility in computing the amount of received information), the test subjects do not distinguish between the vowels ɨ and i. If we consider these vowels as one articulation group, the test subjects will record the vowel i in their replies 2.8 times more frequently than the vowel ɨ. This becomes understandable when we note that in coherent speech the vowel i occurs twice as frequently as the vowel ɨ.

Another example of the effect of linguistic probabilities is the presence of errors made by the test subjects in distinguishing between hard and soft consonants. In order to determine whether the subjects are capable of distinguishing between hardness and softness from visual articulation, we computed the percentage of received information, when this property of consonants was differentiated both at the beginning of a syllable and at the end of a syllable (table 6).

It is clear that the indication of hardness or softness of consonants /627 appearing at the beginning of a syllable was recognized very poorly, and for consonants at the end of a syllable they were recognized even less. The effect of linguistic probabilities could be more pronounced, particularly in this last case--at the end of the syllable when the indication of hardness or softness of the consonant could hardly be recognized visually. In examining the replies it became clear that regardless of whether hard or soft consonants were transmitted, the test subject wrote down hard consonants more frequently (table 7).

It is natural to explain this by the effect of linguistic probabilities of the Russian language. It is known that hard consonants occur three times more frequently in coherent speech than soft consonants (ref. 8).

Furthermore, by examining the intragroup errors of the test subjects in recognizing consonants it was shown that the phonemes which occur more frequently in coherent speech are also selected more frequently by the test subjects. In this case, as we have already indicated, the test subjects prefer to select voiceless consonants more frequently. Perhaps this is partially due to the fact that the voiceless consonants are pronounced by a more simple complex of articulation motions, i.e., without the participation of voice (table 8).

The material obtained shows that the test subjects have information on the frequency of occurrence of various sounds in the Russian language and use this information in their selection. However, it is interesting to establish whether information on the frequency of occurrence is stored in the subject's brain in a form such that a corresponding probability is associated with each phoneme. Apparently the linguistic probabilities are not stored in this form in the memory. However, a person remembers a dictionary of meaningful collections of sounds and their grammatic variations. Since such a dictionary consists of Russian language words, it is natural that its statistical /628 characteristics (the frequency of occurrence of various phonemes in it) must coincide with the statistical characteristics of the Russian language. In other

TABLE 6. PERCENTAGE OF RECEIVED INFORMATION WHEN THE HARD OR SOFT PROPERTIES OF VOWELS ARE DISTINGUISHED.

Test subjects	At beginning of syllable	At end of syllable
G., R., Kh. Recording 1.	9.7	2.4
G., R., Kh. Recording 2.	5.1	0.5
A., S., E. Recording 1.	8.0	0.4

TABLE 7. RELATIONSHIP BETWEEN HARD AND SOFT CONSONANTS IN WRITTEN REPLIES OF TEST SUBJECTS WHEN HARD AND SOFT CONSONANTS OCCURRING AT THE END OF A SYLLABLE WERE TRANSMITTED.

Test subjects	Ratio "hard-soft" when transmitting hard consonant	Ratio "hard-soft" when transmitting soft consonant
G., R., Kh. Recording 1.	2.85:1	1.33:1
G., R., Kh. Recording 2.	2.57:1	1.56:1
A., S., E. Recording 1.	2.70:1	1.86:1
Average	2.77:1	1.58:1

TABLE 8. PERCENTAGE OF CONSONANTS IN GROUPS OF COHERENT SPEECH AND IN THE RESPONSES OF THE SUBJECTS.

	M	P	B	V	F	Ch	Sh	Zh	Shch	L	R
In coherent speech	41	36	23	79	21	32	30	24	14	50	50
In responses	32	48	20	38	62	28	56	12	4	63	37
		N	T	S	Y	D	Z	Ts	K	G	X
In coherent speech		25	23	19	14	11	6	2	61	22	17
In responses		23	46	4	4	21	1	1	57	40	3

words, we can assume that a person does not perform a final phoneme evaluation of each position of the speaker's articulation organs visible to him, but remembers the sequence of these positions. For example, the test subject remembers that the word started with some lip sound followed by a lip vowel and then by some prepalatal sound. The test subject compares this sequence with words stored in his dictionary and selects one of the words which occurs most frequently, for example "pol" (floor).

If a person does indeed react in this way, there must be a pronounced conversion of meaningless syllables into meaningful words. In our investigation this conversion of meaningless syllables into meaningful words took place in a large percentage of cases (table 9).

In the initial tables, the percentage of meaningful syllables was 5.6, while in the replies of the test subjects the meaningful syllable content and meaningful word content reached 61 percent.

The last question, which is of considerable interest and which will be considered very briefly in the present work, concerns the form in which a person memorizes (before carrying out a final decision) the sequence of the speaker's articulation positions which he has observed. We can assume that he remembers this as a sequence of visual images. However references 1, 8-11

TABLE 9. PERCENTAGE OF MEANINGFUL SYLLABLES IN TABLES AND IN THE RESPONSES OF THE SUBJECTS.

Test subjects	In tables	In responses	
		Repetition of individual syllables by speaker were permitted	Repetition of individual syllables by speaker were not permitted
G.	5.6	58.4	33.6
R.	5.6	60.5	49.2
Kh.	5.6	61.1	24.1
A.	5.6	-	36.3
S.	5.6	-	41.1
E.	5.6	-	34.8

and 13 have shown that a person who listens to the acoustic speech signal /629 remembers it not as a sequence of sound samples, but as a sequence of his own articulation movements corresponding to the sounds heard.

Our observations make us believe that during the visual recognition of speech sounds, the same situation occurs. If the test subjects were unable to catch the pronounced sounds at first glance, they immediately repeated the articulation motions of the speaker. If a single repetition did not produce a solution, the test subjects repeated the articulation motions several times, until they were able to select a definite syllable or a definite collection of sounds. Apparently these repetitions of articulations form a definite stage in the process of visual speech recognition.

Everything we have said permits us to make the general conclusion that the visual recognition of speech from the observable manifestations of articulation is determined, first of all, by the supply of words and their grammatic combinations, which are stored in a person's memory and which are necessary for comparison with external signals. With special training a person becomes experienced in "face reading" which, under certain conditions with substantial distortions of speech or with high level of noise, may substantially aid a two-way speech communication.

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THE PROBLEM OF METHODS IN THE FORMATION OF MOTOR HABIT SEQUENCES BY MAN

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The appearance and growth of new professions in which man has the /631
role of a "link in a control system" poses new problems to physiologists and psychologists, requiring penetrating analysis and evaluation of man's higher nervous activity. Such problems include those concerned with information on the rules governing the function and design of information processing systems and control systems of the human brain.

Aboard the spacecraft, in addition to other functions, man performs complex functions of control and communication. This type of activity requires analysis of a complex series of events. A natural desire to simplify this series and make it accessible to analysis has made it necessary for us to use rather crude approximations and to digress from a series of aspects associated with the mechanisms of objective activity. In formulating our problem we proceeded from several general considerations on the functioning of the motor sphere of the human brain, which do not contradict information published in the literature.

We can assume that the realization of complex motor skills which consist of the alternation in some order of various movements is associated with the formation of definite programs for motor activity in the upper regions of the brain. One example of such programs may be the dynamic stereotype studied by I. P. Pavlov and his students. The stereotype is characterized by such features as the completeness of the functional structure and the dependence of the undertaken step on the preceding ones.

The basic feature of the stereotype is the possibility of selecting an alternate step within the action program. Such a program completely deprives its owner of any degree of freedom and converts him into a simple automatic machine. Apparently the motor programs which permit the organism to adjust itself to changing conditions must be based on the probability mechanism of behavior. The organism must have a certain collection of motor acts stored in motor pro- /632
grams, which permit the selection of action most suitable under given conditions.

We proceeded from the concept that the motor programs of man, on the one hand, must have a determined nature and contain some features of the stereotype, and on the other hand must permit him to select the most suitable action at each moment of time. On this basis we made an effort to create an experimental model of such programs.

One such model is considered in the present article. In our experiments the human subject was given the problem of determining a unique sequence of actions by selecting each action from eight possible ones.

For this purpose we used a panel with eight keys and a signal lamp. The test subjects were healthy adults of both sexes in the age group 17-25 (students of the 11th class, college students, coworkers at the Institute). Before the beginning of the experiments the subjects were given the following instructions: to extinguish the signal lamp each time it was turned on with a minimum number of errors. The lamp could be extinguished with any of the keys on the panel. The correct sequence order for operating the keys was determined by a program prepared beforehand, which was characterized by the following features:

(1) Each of the eight keys on the panel was used in the program with the probability of 0.1 or 0.15, i.e., for every 100 signals which turned on the lamp, each key had to be used 10 or 15 times to turn the lamp off.

(2) During a period of 20 cycles (when the lamps were turned on and off) there was no single repeated transition from one key to another. In other words, if we represent two adjoining keys in a program by a diagram, the program had 20 diagrams. After 20 diagrams were transmitted, they were repeated in the same order.

(3) Among the 20 program diagrams there were two groups which, when combined, formed distinct figures (triangles) on the panel.

At the beginning of the experiment the test subject did not know any of the program characteristics. Thus, during the test, he had to learn the following: (a) the presence of regular transitions from key to key; (b) the length of the program and its statistical structure; and (c) the grouping of individual keys and the general order of their investigation.

The pause between the response of the test subject and the following signal was approximately 1 second. The entire experiment lasted 2-2.5 hours with one or two 5-10 minute breaks. During this time 800-1000 signals were produced. The total number of tests necessary to achieve an error-free /633 reproduction of all action sequences was recorded, as well as the average rate of selection, the number and nature of errors.

In processing the experimental results the sequence of replies produced by the test subjects was compared with the fixed sequence, and calculations were carried out to determine information laws caused by errors and the amount of transmitted information determined by the number of coincidences between the program symbols and the response symbols. In all calculations we used simple numbering of the keys (from 1-8). This was true for the program synthesis, its assignment by the experimentator and the recording of responses made by the test subjects.

It was shown by this series of experiments that it is possible to reproduce the sequence of operations with the keys without errors. Eighteen test subjects participated in the experiments. Each test subject was permitted to carry out any number of searches to find the "correct" key and to carry out the search in any order.

TABLE 1. LEARNING PERIOD FOR REPRODUCTION OF SEQUENCE WITH 20 TRANSITIONS USING PANEL WITH 8 KEYS.

Test subject	Age in years	Errors during learning process		Time for first selection (in sec) during learning		Learning rate	
		At beginning	At end	At beginning	At end	According to No. of transmitted signals	According to No. of repeated sequences
M. N.	17	0.80	0	1.8	1.5	160	8
N. P.	24	0.85	0	1.4	1.0	200	10
G. M.	24	0.80	0	3.7	1.3	300	15
L. F.	17	0.80	0	1.2	1.0	360	18
N. N.	17	0.80	0	2.3	1.4	440	22
G. M.	22	0.90	0	1.4	1.3	500	25
V. P.	25	0.85	0	1.4	1.5	560	28
V. M.	17	0.85	0	1.9	1.8	600	30
L. T.	24	0.90	0	1.4	1.3	600	30
V. P.	17	0.85	0	1.6	1.4	700	35
V. V.	24	0.90	0	1.5	1.2	740	37
A. Ch.	17	0.90	0	1.2	1.0	1000	50
L. P.	17	0.80	0	1.4	1.1	1000	50
P. Sh.	24	0.95	0	1.0	2.0	1160	58
O. K.	19	1.0	0			1300	65
O. B.	17	1.0	0	1.0	1.2	2000	100
V. S.	19	0.8				3000	150

Table 1 shows the results of this teaching process. We can see from this table that all 15 test subjects learned to reproduce the program without error. The program consisted of 20 transmissions from key to key, and on the average required 34 repetitions of the sequence. The sequence reproduction level at the end of the teaching process is shown on the graph "P errors" and "signal-response," where "0" means that there is noncoincidence between the specified key and the one selected by the test subject.

During the first transmission of the sequence all test subjects carried out the large number of search key depressions (on the average the correct key was found after the 4th search). The average value of this probability is equal to 0.73. This means that only one of four subsequent choices made by the subject coincided with the assigned key. The time required for each test subject to make one selection at the beginning and at the end of the teaching process as a rule increased somewhat, but not for all test subjects. The average selection time varied from 1.6 sec at the beginning of the teaching process to 1.3 sec at the end of the process.

Redistribution of Errors

A feature to be noted is the large difference in the time required by various test subjects (from 8-100 repetitions) to reach a level of error-free

reproduction of the sequence. It is natural to question the reason for this difference. One of the possible explanations is that in cases of this type the problem is solved by different methods, and the effectiveness of these methods is not the same. However, the analysis of errors produced by the test subjects during the teaching process showed that the methods of learning for various people were similar in principle.

Eight test subjects were selected so as to combine those which were slow to learn and those which were fast to learn. For these eight the sequence /634 of response reactions was analyzed during the first six transmissions of the fixed program. Table 2 presents the data which characterize this group of test subjects.

We see from the table that the group included people who learned very rapidly (10 transmissions) as well as those who learned slowly (150 transmissions). At the same time, the initial period of learning for all test subjects contained common features. The average number of selections for a single transmitted signal during the first six transmissions remains quite large. Only in the case of subject N. P. did the number of selections decrease by 50 percent of the initial level after five transmissions. For the remainder of these subjects, this decrease was insignificant and the average number of selections after five transmissions was greater than three.

When the number of selections is expressed in the form of the /635 average probability of noncoincidence (fig. 1, upper curve) and when the numbers obtained in a group are averaged, it turns out that in this stage the learning curve drops very insignificantly and is extremely flat. This would seem to indicate that no learning takes place during this stage. At the same time the analysis of errors which are incurred during this period shows that there is a redistribution of the sequence of responses. During this time certain errors begin to disappear while others increase. In figure 1 the lower curve shows the probability of selection by the test subjects of such key combinations which are "not valid" for the given problem. The middle curve shows the probability

TABLE 2. PROBABILITY OF ERRORS IN INITIAL PERIOD OF LEARNING.

Test subject	Rate of learning according to quantity of programmed transmissions	Probability of error number during first transmissions of program						
		1	2	3	4	5	6	7
N. P.	10	0.85	0.75	0.6	0.55	0.5	0.2	0.2
G. M.	25	0.9	0.7	0.65	1.0	0.6	0.7	0.7
V. M.	30	0.85	0.85	0.7	0.8	0.75	0.65	0.8
L. T.	30	0.9	0.65	0.8	0.75	0.8	0.7	0.75
V. V.	37	0.9	0.85	0.95	0.85	0.8	0.8	0.9
S. K.	65	1.0	0.85	0.85	0.65	0.7	0.6	0.6
O. B.	100	1.0	0.95	0.85	0.9	0.8	0.8	0.75
V. S.	150	0.8	0.8	0.8	0.8	0.7	0.75	0.8
Average		0.9	0.8	0.77	0.79	0.7	0.65	0.68

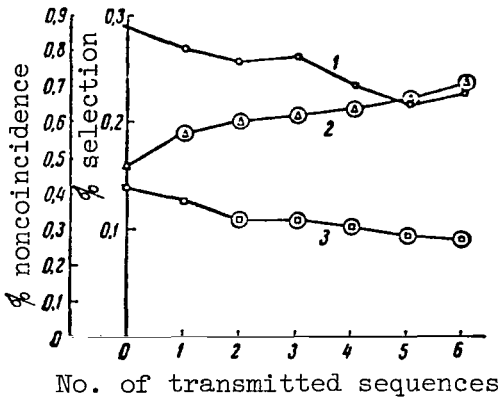

Figure 1. The probability for the selection of "valid" and "invalid" combinations during the initial training period (average data, eight test subjects). 1--percentage of noncoincidence in the "signal-response;" 2--percentage of selection of "valid" key; 3--percentage of selection for the "invalid" key. The circled points are for the level of significance equal to or less than 0.01.

of selecting combinations which are "valid" for the given problem. The nature of the curves clearly shows that one transmission of the sequence is sufficient to change the nature of the responses and for the random nature of the search to cease.

Here it is appropriate to clarify the "valid" and "invalid" combinations for a given problem. In the quantitative evaluation of some sequence as a first approximation, it is customary to note the frequency of the elements of which it consists (in our case, these will be the individual keys). In the second approximation the interrelation of all "neighboring" elements is evaluated: the total number of different combination pairs is computed.

It is precisely the combination pairs of this type that were noted by us when they were subdivided into "valid" and "invalid." It is known that eight elements can be used to develop 64 combination pairs. In our program we used only 20 combination pairs. These were the ones that were considered "valid." In addition, we considered 10 more combination pairs as "valid" in our problem. They are associated with one specific error of the given problem (the error associated with the inaccurate reproduction of definite pieces of the sequence). Thus, the number of "valid" combinations in the given problem was assumed to be equal to 30, while the number of "invalid" combinations was taken to be equal to 34.

In analyzing the responses of the test subject each step of each search was examined and was evaluated to determine whether it belonged to the first group ("valid") or the second group ("invalid"). Then the information was reduced to one average probability of the "valid" key selection during each step.

The reasoning in this case was as follows. If a person knows nothing /636
about the structure of the problem, then during each step, on the average, he selects any of the eight keys with the same probability, which is equal to 0.12. In this case, in approximately half of the cases (30 out of 64) he will select the "valid" keys. Thus, the probability of selecting one "valid" key under completely random conditions is equal to 0.12. The probability of selecting the "invalid" key is also approximately the same (if we do not try to be too exact). The deviations from this level can certainly be looked upon as deviations from the random process.

In our case, we show in figure 1 the deviations of the curves in the direction of an increase in the probability of selecting the "valid" keys and a drop in the probability of selecting the "invalid" keys. The magnitude of the deviation from the first point was computed by using criterion X^2, and the significant increase in the curve ($P < 0.01$) for the probability of selecting the "valid" keys was already established after the first single transmission of the sequence (the points for which $P < 0.01$ are circled). The decrease in the probability of selecting the "invalid" keys, which was significant at the same level, was detected after two transmissions of the sequence.

Grouping of Responses

We briefly consider one type of error which is characteristic of the given problem. These errors are related to the spatial structure of the problem. The fact is that in the assigned sequence, if we consider its drawing /637
(drawing showing the motion of the hand over the panel), we detect two accumulations of points which form a closed outline--a triangle (fig. 2A). These outlines turn out to be the first signs of the sequence adopted by the test subjects. This is expressed in the fact that the test subjects, having discovered the region of the closed outline, give preference to the selection of points which supplement the drawing of the triangle. However, the direction and order of selection of these points frequently do not correspond to the unique order which is assigned and change continuously, as shown in figure 2B. The test subjects appear to carry out the search inside the outline. This type of behavior is very similar to the "local search" described by Gel'fand and Tsetlin (ref. 1) as one of the methods of expedient behavior. If we take into account all combination pairs, which occur when the outline of the triangle is followed in the direction contrary to the one assigned, they form those additional 10 "valid" combinations mentioned above.

In considering the responses of the test subject it was noted that at the very beginning of the learning process (after one or two transmissions of the sequence), the errors changed their nature not only by decreasing the probability of "invalid" combinations, but also by forming individual groups characterized by common criteria. The error-free responses also occurred in groups.

As a general tendency, we note that the learning process did not represent a gradual decrease in the number of selections for each point, but was rather characterized by nonuniformity. In this case there were large groups of error-free responses (5-8) and of responses realized with a maximum search.

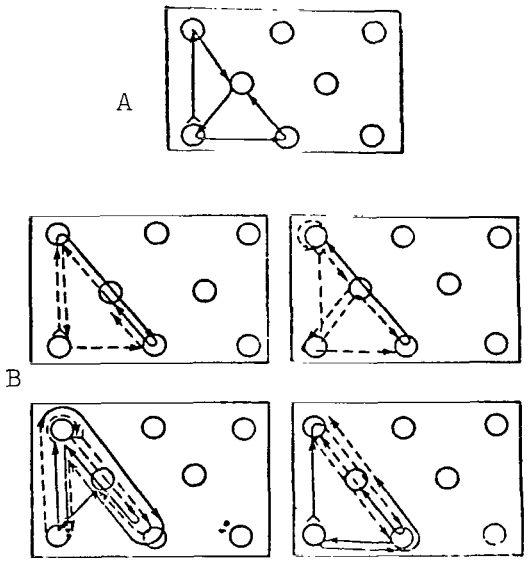

Figure 2. The formation of closed contours in the 20 component sequence. A--assigned configuration; B--configuration traced during the learning process. The solid lines show transitions to the "valid" key while the broken lines show the wrong transitions.

The subsequent analysis was directed to clarify the size of a group of responses which is assimilated most rapidly by the test subject and is realized either in the form of error-free actions or in errors. These groups were /638 examined according to three criteria: error-free groups of responses; groups of responses which represented pieces of the program but not in the right place; and a group of responses representing those pieces of the program which form clear figures (triangles), including those triangles which are traced in the direction contrary to the one assigned.

The data of this analysis are shown as curves in figure 3 showing the deviations in the number of observed groups for 8 test subjects (table 2) with a significance level no greater than 0.01. The curves were drawn in the following manner: the number of response groups was computed (for each of the three criteria) during the first transmission of the program and this number was taken as reference. Subsequently the number of these groups in each following transmission of the program was computed and compared with the reference using the matching of X^2 as a criterion. Those points where the significance level was equal to 0.01 were plotted on the curve.

Figure 3 shows that (a) the magnitude of the group which is assimilated most rapidly by the test subject is equal to 3; (b) assimilation by groups of three characterizes both learning in accordance with the criterion of figures produced by the sequence of actions, as well as the learning of the exact sequence of a given program; (c) to assimilate such groups of responses according

to the figure criterion requires one transmission of the program; (d) assimilation of a group of responses associated with a given program (without arranging them in their places) requires two transmissions of the program; (e) error-free reproduction of such groups of responses requires four transmissions of the program; and (f) in all cases the reproduction of groups consisting of four responses requires a large number of program transmissions meant for groups consisting of three responses.

In evaluating the results of the analysis of replies produced by the test subjects, we get the impression that the first period of learning to reproduce the assigned sequence of actions takes place in the same manner for different test subjects. During this period the quantity of errors does not vary significantly, and a regrouping of the responses takes place. This stage is exhibited by all persons during a period of several (up to 600) initial transmissions of the programs. The entire remaining learning period (which differs in duration for different subjects) represents a rearrangement of assimilated groups and the refinement for the position of individual actions inside a group.

Thus, the conditions of the problem which had to be solved experimentally posed many diverse requirements to the test subjects. Since the direction of each individual motion of the hand over the panel was not designated by a natural control signal, it was necessary to find reference points for carrying out the error-free selection. There were 20 such individual directions in the problem, and these 20 directions of individual action had to be selected from 64 possible ones. After isolating all directions corresponding to the problem, they had to be arranged in one unique assigned order.

Such a problem required the organization of actions into a system and /640 obviously had to be solved in stages. The analysis of the sequence of responses had shown that at the very beginning of the learning process, the test subjects regrouped their actions in the direction of producing 3 groups corresponding to the spatial structure of the program. This type of regrouping can be rightfully considered in the light of Miller's concept (ref. 5) on excessive coding. This concept means that by using an enlargement of the code symbol it is possible to increase the carrying capacity of a channel. In this case the symbol carries a large load, but since the carrying capacity is limited only by the number of symbols, there is an overall gain in the quantity of processed information.

We are convinced that Miller's concepts can be applied to the present experiment also by the fact that after grouped responses were reproduced by the test subjects, the utilization of these groups as an entity was observed. Thus, if the error included a symbol already associated in a group, it was followed by the remaining members of this group. The error was therefore enlarged, but the test subject did not notice this and repeated the same errors during the next step. However, this also made it possible immediately to realize a group of error-free responses when the "correct" place was selected.

Similar data were obtained in an experiment where 26 letters arranged in a random fashion were presented. The process of memorization occurred in stages of grouping (most frequently grouping in threes). This permits us to assume that this method of organizing a problem by man has a general nature (ref. 2).

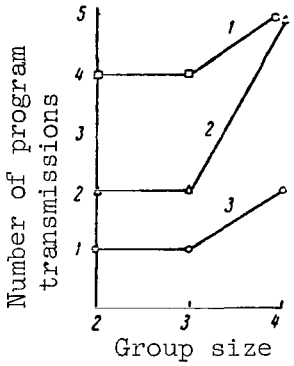

Figure 3. Rate of appearance of response groups as a function of group size and grouping features (average data, 8 subjects). 1, error-free responses; 2, displaced part of program; 3, groups by "triangle." Each point has level of significance ≤ 0.01.

The question of the arrangement of groups in their correct places has not been sufficiently analyzed by us. For unclear reasons, people expend a different amount of time for this process. Whereas it takes one to two transmissions to regroup the responses, it takes 100 or more transmissions for some test subjects to arrange the groups according to their places. At the same time there are test subjects who were able to carry out this process during 5-10 transmissions. If we neglect these variations and turn to the results of the teaching process, then experiments show that man is capable of learning the error-free reproduction of a sequence of actions consisting of 20 transitions by selecting each step from 8. Man apparently builds his own program of actions, which is abstracted from his concrete motions and may be realized as a whole in a new, changed situation.

After the teaching process all test subjects were presented with a text in which the sequence was changed so that it was a mirror image of the initial program. We shall not consider the results in detail since they will be published in a special communication. We only note that the test subjects were able to identify the sequence which they learned. This was expressed both in the oral evaluation of the problem as well as in the nature of action. The oral evaluations were as follows: "Everything is being done backwards," "everything is inverted," "they are running it backwards." One test subject asked whether he could invert his panel.

The speed of solution of this problem compared with the first one was very high at the high level of significance (≤ 0.02), and on the average constituted 11 transmissions. Individual test subjects correctly reproduced the inverted sequence after one or two transmissions.

Behavior of this type causes us to adopt the viewpoint of those authors who developed the concept that by the mechanisms of abstraction and the transposition of rules a person learns more than what he is taught (refs. 3 and 4).

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AUTOMATIC ANALYSIS OF DIURNAL PERIODIC CHANGES IN HUMAN ELECTROENCEPHALOGRAM

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The study of the dynamics of change in an electroencephalogram /642
stemming from the impact of external environmental factors on the organism requires that we take into account daily fluctuations in the bioelectric activity of the brain. The literature provides us with data on daily periodicity of EEG obtained primarily from patients in psychiatric clinics (refs. 5, 6, 8). All investigators who have studied daily EEG changes in healthy persons have done so primarily in connection with nocturnal sleep (refs. 1-4 and 7). These authors restricted themselves to visual analysis. Our research utilized automatic analysis of EEG for determining diurnal fluctuations in the bioelectric activity of the brain in healthy persons. Tracings were made four times daily for 10-30 days of the EEG of 6 healthy men between the ages of 20 and 23 (at 10 a.m., 5 p.m., 1 a.m. and 5 a.m.). Discrete EEG tracings of nonsleeping subjects were recorded at night. Bipolar bioelectric potentials of the brain were taken from the frontal and occipital regions. In order to determine the subjects' overall condition, simultaneous recordings were made of the EKG, pneumogram and arterial blood pressure. An important advantage of the applied method was the frequency analysis of the EEG with a periodmeter, following separation of individual rhythms with a band-filter analyzer. Analyses were made of Δ, θ, α, and β_H rhythms (14-20 cps). The periodmeter determined the frequency characteristics of each EEG wave, as a result of which it was possible to establish the number of oscillations of a specific frequency per unit of time. The distribution of waves according to frequency enabled us to detect frequency variations within each physiological rhythm of the EEG. The composite EMS (composite bioelectric intensity) of the EEG, as well as the EMS of individual biocurrent rhythms were calculated on an integrator /643
(in relative values). A total of 295 frequency spectrograms of the EEG were studied and 467 computations made of EMS.

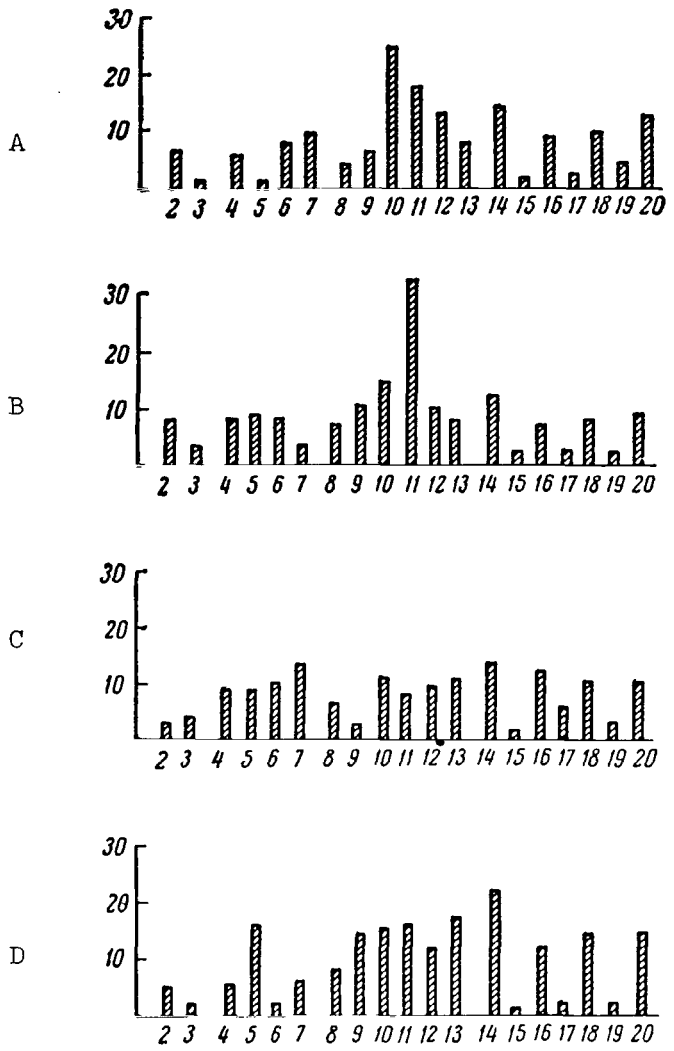
Frequency analysis of EEG showed that the EEG of healthy persons in the waking state always contain Δ and θ waves (spectrogram, parts A, B and D). These could not be detected by visual analysis, because they had been obscured by the more frequent α and β_H rhythms. These findings cast doubt on the old idea that Δ and θ waves appear only in an EEG during deep inhibition of the central nervous system (narcosis, sleep) or a pathological condition of the organism (hypoxia, mental disorders, coma, etc.). The detection of Δ and θ waves on an EEG under such conditions is related to an increase in the amplitude of slow oscillations and also quite probably to a decrease in general cortical

α and β activity. In consequence, Δ and θ rhythms are always present in an EEG recorded either during the waking or sleeping state. However, it is not the modification of these rhythms which determines the general appearance of an EEG over the course of 24 hours. It has been found that the most characteristic feature of the EEG for a 24-hour period is the change taking place in the α rhythm. The majority of the spectrograms of the α rhythm obtained at 5 p.m. showed displacement of the dominating frequency of the α rhythm by higher frequencies (by 1-2 cps) as compared with morning tracings (B). The spectrograms traced during sleep lacked the clearly defined maximum α waves of specific frequency so characteristic of daytime α rhythm. Instead, the relative quantity of lower frequency α waves showed an increase (leftward frequency shift of EEG). The α waves were rather evenly distributed over the entire breadth of the filter for passing through α waves of 8 to 13 cps (C). The total number of α waves decreased as compared with daytime tracings of EEG. Spectrograms of the α rhythm of nonsleeping subjects recorded at night resembled the spectrograms made at the end of the work day (5 p.m.) or in some cases the EEG in a state of sleep with respect to the distribution of frequencies of the α rhythm (D).

The β_H rhythm was distinguished by the absence of a clearly dominating frequency. During sleep the total number of β_H waves decreased, as compared with daytime tracings. The relative number of β_H waves in the EEG of some patients increased towards evening. In general, the β_H showed less dependence than the α rhythm on the time of day.

As indicated in the beginning, Δ and θ waves are constantly present in the EEG. The total number of Δ and θ waves isolated by the analyzer within the filters' limits for these frequencies is frequently several times smaller than the total number of α and β_H waves. The number of these waves fluctuated within wide limits: Δ waves from 1-15, θ waves from 15-56 per 10 sec. No correlation could be established between the time of the day and the number of Δ and θ waves (A, B, C and D). /645

The EMS for the entire EEG, as well as for the isolated θ, α and β_H rhythms, was of fairly constant size for any particular time of the day. The smallest EMS for an EEG was in the morning, the largest at night during sleep. The EMS for an EEG in the evening was as a rule higher than the EMS for morning tracings. The EMS increased towards evening with both open and closed eyes. The degree of the reaction of the EMS to the closing of the eyes was more marked, varying from 143-300 percent of the EMS with eyes open. The EMS for the θ and β_H rhythms upon closing of the eyes changed very little or not at all. This makes it possible to assume that the basic reason for changes of the EMS of an EEG



Spectrogram of EEG of subject F (female), recorded at 10 a.m. (A), 5 p.m. (B), 1 a.m. while asleep (C) and 5 a.m. while awake (D). Height of columns (in mm) corresponds to number of waves of particular frequency. (Numbers under columns indicate frequency in cps (2, 3, Δ rhythm; 4, 5, 6, 7, θ rhythm; 8, 9, 10, 11, 12, 13, α rhythm; 14, 15, 16, 17, 18, 19, 20, β_{II} rhythm.)

during the waking state lies in changes of the EMS for the α rhythm. The increase of EMS during sleep does not stem from an increase in the number of Δ and θ waves, but from an increase in their amplitude.

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EXPERIMENTAL CONFINEMENT OF FISH IN HERMETICALLY SEALED AQUARIUMS WITH AND WITHOUT CHLORELLA

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A closed ecological system may include among its components a chain /646 consisting of bacteria--algae--zooplankton--fish. The links making up this chain may be maintained either individually or in combination. The most practical method would be to maintain them jointly in the limited cabin space of a spacecraft.

The present work proposes to elucidate the possible joint maintenance of individual components of the chain in a limited volume of water in a hermetically sealed aquarium. The first stage of the investigations involved the selection of two basic links (fish and algae--Chlorella). In this connection it was necessary to establish several facts. With respect to joint maintenance, it was necessary to determine the lifespan of the fish in a hermetically sealed aquarium containing Chlorella at a specific concentration as a source of oxygen and food. For separate maintenance it was important to consider the Chlorella solely as a source of oxygen. It was also important to determine the effect on this index of temperature and illumination, volume of water, density of vegetation, etc.

A number of reasons caused us to select Prussian carp for the experiment. This species is distinguished by a relatively low level of oxygen consumption, by its ability to live in relatively muddy water and by the ability of its gills to filter plankton.

Two species of Chlorella served as sources of oxygen and food--Chlorella vulgaris 25 and Chlorella pyrenoidosa 82.

For the experiment we used one-year- and two-year-old carp, which had been kept in a special tank before the experiment.

Both Chlorella species had been grown in the laboratory in a special /647 arrangement, using culture dishes and ventilation with a mixture of oxygen and carbon dioxide. Chlorella pyrenoidosa 82 was grown on a Tamiy medium and Chlorella vulgaris 25 on a modified Meyers medium. Before being put in the aquarium with the fish, the Chlorella was first removed from the medium, washed twice in tap water and then centrifuged.

The experiments were conducted in two types of aquariums, made of plexiglass. The first type was a rectangular vessel, measuring 58 x 20 x 19.5 cm. It had a partition 6 cm short of the top, dividing the aquarium into two compartments, one being 8 liters in volume, the other 16 liters. The top had three openings: an intake with a screw-on cap and two openings for the insertion of stoppers--one with a thermometer and tube for removing water samples and the other with a separatory funnel and tube. A rubber sac was stretched on the tube and immersed in the aquarium for maintaining a constant water volume to compensate for the removal of samples.

The second type of aquarium was a square reservoir with a conically shaped bottom containing an opening for a water outlet. The top of the aquarium had the same type of design as the first type. The volume of the second type of aquarium was 9.8 and 29.8 liters.

All openings of the aquariums were hermetically sealed and the seal was checked later. The aquariums were illuminated by natural light during the day and by luminescent lights around the clock. The surface of the aquarium received an average illumination of 2,550-3,800 lx.

The aquariums were filled with fixed amounts of settled tap water, then Chlorella in the required concentration was added and after that the previously weighed fish. The water was tested for oxygen and carbon dioxide content and the initial concentration of Chlorella. The aquariums were then sealed hermetically. Throughout the course of an experiment the temperature was taken and the behavior of the fish observed three times daily. The length of each experiment was determined by how long the fish lived. The final concentrations of oxygen, carbon dioxide and Chlorella were measured after the fish died without disturbing the hermetic seal of the aquariums.

Two series of experiments were carried out. In the first series, the fish were kept in aquariums of the first type, separate from the Chlorella. The volume of the compartment with the Chlorella was 8 liters, that with the fish 16 liters. Both compartments had a common air cushion through which diffusion of gases operated in both directions. In this series, the fish were supplied with oxygen produced by Chlorella by photosynthesis, but were not allowed /648 to feed on the Chlorella, which, in this series, was Chlorella pyrenoidosa 82.

In the second series, the fish were kept in aquariums of the second type together with Chlorella. The air cushion was absent. The cells of the Chlorella provided the fish with oxygen and also served as food for them. Chlorella vulgaris 25 was used in this series. Comparable aquariums were used as controls for each of the series. They contained fish under the same conditions, except that they did not contain Chlorella and were not illuminated by artificial light.

In a number of cases, where it was necessary to evaluate the significance of the difference between the initial and final data, statistical processing was done. The methods used were those developed for small selections ($n < 30$). Evaluation of the significance of the difference was made within the framework of a 5 percent level of significance by determination of Student's t criterion. The found value for t was compared with the table value $t_{0.05}$ for f degrees of

TABLE 1. SURVIVAL OF PRUSSIAN CARP IN AQUARIUMS WITH PARTITIONED COMPARTMENTS.

Water temperature in compartments, °C	Compartment with fish						Compartment with Chlorella			
	No. of fish	Total weight of fish, g	Density of planting, g/l	Oxygen content, mg/l	% of saturation with oxygen	CO_2 content, mg/l	Chlorella cell concentration in 1 ml of water, millions	Oxygen content, mg/l	% of oxygen saturation of water	Duration of survival of fish, days
25.2	5	101.8	6.37	8.03	93.5	10.5	Het			9
26.6				1.03	12.3	25.03				
26.2				0.99	11.7	55.00				
24.6				1.00	11.5	66.00				
27.0	4	96.0	6.00	8.00	95.8	12.10	3.00	14.00	167.8	39
28.0				1.15	14.0	40.70		22.50	273.7	
24.0				1.62	18.5	37.40		14.50	165.5	
27.5				1.44	17.4	29.70		40.50	489.1	
28.5				1.50	18.4	44.00		27.10	332.1	
27.0				1.40	16.8	49.50		47.30	566.5	
24.5				2.60	29.9	52.80	4.52	20.40	234.7	
24.5	5	102.5	6.41	8.00	92.0	15.40	5.42	7.60	87.4	49
27.0				0.51	6.1	66.00		33.90	406.0	
28.5				0.81	9.9	56.40		30.70	376.3	
27.4				1.12	13.5	48.40		30.24	364.3	
23.5				2.30	26.0	47.30		30.90	349.9	
27.3				1.60	19.2	37.40		24.60	296.0	
28.2				1.80	21.9	38.50		26.60	324.4	
27.0				1.53	18.3	49.50		27.30	326.9	
24.4				1.95	22.4	58.30	5.50	18.30	210.3	

freedom. The difference was considered as significant when $t > t_{0.05}$ and as insignificant when $t \leq t_{0.05}$. For graphic representation, values are given in table 2 for $t/t_{0.05}$ which, from the above, would be > 1 in the case of a significant difference and ≤ 1 in the case of an insignificant difference.

The results of the investigations are presented in tables 1, 2 and 3.

Table 1 contains the results of the first series of experiments, which was used to study the survival of Prussian carp in a hermetically sealed aquarium, divided by a partial partition into two compartments. One of the compartments contained the fish, the other a suspension of Chlorella. Tables

TABLE 2. CHANGE IN CONTENT OF OXYGEN AND CARBON DIOXIDE GAS IN HERMETICALLY SEALED AQUARIUMS DURING MAINTENANCE OF CHLORELLA AND PRUSSIAN CARP.

Series of experiments	Aquarium No.	No. of Chlorella cells per ml		Water temperature, °C		Oxygen content of water				Carbon dioxide gas content of water, mg/l			
						mg/l		% of saturation		Free		Carbonate	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
I	1	6.73	1.33	18.0	25.0	9.60	0.51	98.6	5.9	21.56	(17.60)	0.00	(0.00)
	2	6.67	8.95	18.0	25.0	9.60	1.40	98.6	16.2	21.56	0.00	0.00	19.80
	3	6.15	18.85	18.0	26.0	9.60	0.64	98.6	7.5	21.56	0.00	0.00	19.80
	4	7.54	1.41	18.0	25.0	8.10	0.64	83.2	7.4	28.60	0.00	0.00	22.00
	5	7.08	17.42	18.0	27.5	8.10	0.74	83.2	8.9	28.60	0.00	0.00	26.40
Average		6.83	9.56	18.0	25.7	9.00	0.80	92.4	9.1	24.40	0.00	0.00	22.00
II	1	6.00	9.13	25.0	25.3	5.44	0.76	63.1	8.8	14.08	0.00	0.00	26.40
	2	6.00	9.09	25.0	28.0	5.40	0.71	62.6	8.6	14.08	0.00	0.00	38.80
	3	6.00	8.07	25.0	27.0	6.14	(4.71)	71.2	(56.4)	14.08	0.00	0.00	26.40
	4	3.00	5.70	25.0	32.0	6.77	0.44	78.5	5.7	12.32	(8.80)	0.00	(0.00)
	5	3.00	0.80	25.0	27.0	6.57	0.74	76.2	8.9	15.40	0.00	0.00	30.50
Average		4.80	6.56	25.0	27.9	6.06	0.66	70.3	8.0	13.99	0.00	0.00	28.5
Aquariums without Chlorella		25.0	25.0	6.87	0.75	79.7	8.7	17.04	31.68	0.00	0.00		

Note. The amounts shown in parentheses were not used in computing averages because of their sharp deviation from the sample.

TABLE 3. SURVIVAL OF PRUSSIAN CARP IN HERMETIC AQUARIUMS CONTAINING CHLORELLA.

Series of experiments	Aquarium No.	Size of aquarium, liters	No. of fish	Total weight of fish in aquarium, g			Density of planting, g/l	Length of survival, days
				Initial	Terminal	Significance of difference between terminal and initial weight, $t/t_{0.05}$		
I	1	9.8	2	162.23	167.80		16.55	14
	2	9.8	2	156.00	192.10		15.92	15
	3	9.8	2	148.84	172.30	0.43	15.18	13
	4	29.8	4	314.28	320.50		10.55	11
	5	29.8	4	306.18	330.60		10.27	25
Average				217.51	236.68		13.70	15.6
II	1	9.8	1	82.90	83.50		8.46	24
	2	9.8	1	80.90	80.50		8.25	30
	3	9.8	1	81.85	82.90	0.03	8.35	14
	4	29.8	3	220.30	214.85		7.21	37
	5	29.8	3	197.01	197.74		6.63	25
Average				131.65	131.86	-	7.80	26.0
Aquarium without Chlorella		5.14	2	61.50	61.50	-	11.97	1

2 and 3 show the results of the second series of experiments, which investigated the survival of Prussian carp in a hermetically sealed aquarium containing Chlorella.

Before analyzing the results of each series, it was essential to examine the survival of the fish in the control aquariums, in order to use these data in the future as an original background for comparison with experimental aquariums.

As might be expected, the fish in the control aquariums, deprived of a continuing supply of oxygen, died much sooner than did those in the experimental aquariums. We draw attention to the fact that the fish died within the first day of the experiment in the hermetically sealed aquariums without an air cushion, but survived 9 days in those provided with air cushions. In the first case, death took place as a result of a greater oxygen deficiency than in the second. There is no doubt that such a big difference in survival time of the fish could have been due to individual differences among the fish. Other

factors to be taken into account include the size of the fish, density of planting (11.97 g/l against 6.37 g/l in aquariums without an air cushion). The supposition that the presence of an air cushion over the surface of /649 the water prolongs survival of fish in aquariums is completely reliable.

Analysis of the results of the experiments conducted in aquariums in which the fish were separated by a partition from the Chlorella discloses perfectly consistent dynamics for oxygen and carbon dioxide dissolved in the water (table 1). In the compartment containing the fish there was a sharp drop in /650 oxygen content and an increase in carbon dioxide content during the first two days of the experiment. In one case, after a lapse of two days, the oxygen content dropped from 8.00 to 1.15 mg/l and in another to 0.51 mg/l, i.e., to that threshold level below which fish begin to die.

However, the oxygen content subsequently increased and to the termination of the experiment, i.e., until the fish died, stayed at a level of 1.40 to 2.60 mg/l, corresponding to 16.8-29.9 percent saturation of the water with oxygen. The carbon dioxide content of the water increased on the third day of the experiment from 12.10-15.40 mg/l to 40.70-66.00 mg/l, after which it remained at a level in the range of 29.70 to 58.30 mg/l to the end of the experiment.

Such a constancy in oxygen and carbon dioxide content of the compartment with the fish, symptomatic of gas exchange, clearly shows natural diffusion of gases through the air cushion connecting the compartment with the fish to the compartment with Chlorella. A relative constancy as to content was also maintained, following the sharp increase of oxygen content in the compartment with Chlorella. The data of the first series of experiments thus clearly point to the diffusion of oxygen through the air cushion from the compartment with the fish and to the diffusion of carbon dioxide in the opposite direction. We cannot yet determine the intensity of this diffusion, but we can assume that it is relatively small, inasmuch as saturation of water with oxygen in the compartment with the fish remained at a low level, no higher than 29.9 percent of full saturation of the water at the given temperatures.

It is of great practical interest to calculate how much oxygen is supplied to the experimental fish by Chlorella from photosynthesis. The optimal level of oxygen consumption by Prussian carp at 16°C is on the average 0.066 mg O_2 per hour per gram of live weight of fish. Converted to the actual temperature of the experiment (27°C) with the help of Vinberg's temperature correction, this level becomes on the average 0.142 mg O_2 per hour per gram of live weight of fish. On this basis, we find that optimal consumption of oxygen by fish during the first experiment should have been 12.76 g for 39 days and during the second experiment 17.12 g for 49 days. To what extent then did the Chlorella supply the oxygen requirements for Prussian carp? Taking 4 mμ to be the average size of Chlorella pyrenoidosa, the dry-matter content of whose cells was no more than 10 percent, we computed the dry weight of the Chlorella cells in the experimental aquariums. We found that photosynthesis produced 9.58 g of oxygen in 39 days during the first experiment and 16.86 g during the second experiment.

Prussian carp were thus supplied with 77.2 percent of optimal oxygen /651 requirements in the first experimental aquarium and with 98.5 percent in the second. These data are orientative and approximate. Nevertheless, they agree rather well with the experimental results obtained regarding survival of the fish. Thus, the fish survived 39 days in the aquarium where they received a smaller supply of oxygen as opposed to 49 days in the second aquarium.

These data allow us to estimate that, within the framework of these experiments, Prussian carp would receive a normal supply of oxygen where the density of the fish population (in g/l) would about equal numerically the concentration of Chlorella pyrenoidosa 82 cells (in million/ml).

The second series of experiments (tables 2 and 3) investigated the length of survival of the fish in joint maintenance with Chlorella and the changes in the live weight of the fish, the gaseous content of the water and the content of Chlorella cells in aquariums. In comparing this series with the preceding series of experiments, attention is drawn to the briefer survival of the fish in aquariums of the second series, which did not contain an air cushion over the water. This fact which, as indicated above, had also been observed in the control aquariums clearly points to the positive value of the air cushion in hermetically sealed aquariums for increasing the length of survival of the fish.

In aquariums with an air cushion, the fish survived 39-49 days and in aquariums without an air cushion only 11-37 days. It is interesting to note that, in preliminary experiments conducted to work out a methodology, Prussian carp survived 45 days in aquariums with an air cover and joint maintenance with Chlorella.

During the second series of experiments, the dynamics of the values under study were determined on the basis of initial and terminal indexes. Completely consistent changes in these values were observed here also. For example, it may be pointed out that the fish died, with two exceptions, in oxygen concentrations of 0.44-0.76 mg/l which corresponds to 5.7-8.9 percent of full saturation of water at the given temperatures. A sharp decrease in oxygen content towards the end of the experiment indicates quite clearly the low level of photosynthesis by Chlorella in the experimental aquariums. The oxygen produced by the Chlorella was manifestly insufficient for normal respiration of the fish, especially since a considerable part of the oxygen went into processes not connected with respiration (oxidation of fish excreta and of organic substances produced and released into the water by Chlorella cells, the actual respiration of the Chlorella cells and oxidation of products of saprophytic bacteria associated with Chlorella cells).

We consider the basic reasons for the weak operation of photosynthesis in Chlorella during the second series of experiments to be the following:

(1) inadequate illumination due to the energetic growth of Chlorella in aquariums and, in this connection, the increase in optic density of the Chlorella suspension;

(2) inadequate supply of carbon dioxide to Chlorella cells, fish being the fundamental producers of the gas in these experiments.

It was thus impossible in the second series of experiments to provide the fish with sufficient oxygen from photosynthesis of Chlorella. This complex problem evidently needs additional research for its solution. It will be necessary to determine an effective ratio between density of fish population and density of Chlorella suspension, taking into account the multiplication rate of Chlorella and the rate of its consumption by the fish. /654

The data of the second series of experiments disclose a definite relationship between the density of fish population and length of survival. For example, when the density of population was 13.7 g/liter, average survival for the fish was 15.6 days, but when the density of population was 7.8 g/liter, survival averaged 26 days. For comparison we point out that density of population for the first series of experiments was 6.0-6.4 g/liter, while length of survival was 39-49 days.

In the second series of experiments, data relating to the change in live weight of fish during joint maintenance with Chlorella were subjected to statistical treatment. The results of this treatment showed that the changes in the weight of the fish observed during the experiment were not significant within the limits of a 5 percent level of significance and could not have been due to the conditions of the experiment. This consideration indicates that during the course of the experiment the fish fed on Chlorella and maintained their weight at the initial level. Feeding on Chlorella is confirmed by the excreta, which contained partially broken down or completely decomposed Chlorella cells.

VARIANT METHOD FOR DETERMINING MAXIMUM CHLORELLA PHOTOSYNTHESIS

Ye. A. Ivanov

Small size and weight are possible for a biological air regeneration /655 system (BARS) based on Chlorella photosynthesis only when the productivity of this oxygen culture is increased to the maximum.

The article describes an attempt to determine the maximum practicably attainable level of productivity based on the processing of experimental data.

For the purpose of simplifying the quantitative relations, it is proposed to introduce into consideration a value characterizing the growth rate of Chlorella cells. Let us call such a value the cell growth tempo (T_p). Its formula, by analogy with Shmal'gauzen's well-known formula, can be expressed as

$$T_p = \frac{1}{n}\frac{dn}{dt}, \tag{1}$$

where n is the density of the cell culture, that is, the number of cells per unit of suspension volume, and t is the time.

Integrating I, where $T_p = T_p(t)$, we obtain the following expression for the current value of the culture density

$$n = n_0 e^{\int_0^t T(t)\,dt}, \tag{2}$$

where n_0 is the density at the moment in time t = 0.

If we introduce the value q to indicate the volume of oxygen released by each cell during the time of its growth, the expression for the amount of oxygen Q produced by the suspension during time t correct to values of the second order of smallness may be written in the form /656

$$Q = Vqn_0\left(e^{\int_0^t T_p(t)\,dt} - 1\right); \tag{3}$$

where V is the volume of the algae suspension.

In such an instance the rate of release of oxygen $\dot{Q}$ will be determined by equation

$$\dot{Q} = Vqn_0 T_p(t)\, e^{\int_0^t T_p(t)\,dt}. \tag{4}$$

During the operation of BARS, the density of the culture n will be maintained at some fixed level that ensures the most favorable conditions for cultivating algae. Other things being equal, the tempo of cell growth T_p will remain unchanged and, in consequence, equations 2, 3, and 4 will assume the form

$$n = \text{const} \tag{5}$$

$$Q = qVT_p nt \tag{6}$$

$$Q = qVT_p n. \tag{7}$$

The value of q changes relatively little following any change in the conditions of cultural growth. Experience shows that it lies usually within the limits of $(0.8 - 1.5)\cdot 10^{-8}$ cm^3 per cell. In this connection, the low values of q correspond more often to the more intense cultures and vice versa.

If we remember that the specific productivity of the culture is $\dot{Q}_y$, i.e., productivity per unit of volume of the cell suspension, we obtain its maximum value from the equation

$$\dot{Q}_{y\,max} = q\,T_{p\,max} n_{max}. \tag{8}$$

In order to determine the maximum practicably attainable value for the quantity T_p, it was necessary to process the results of experiments in growing Chlorella in flat pans 40 mm deep. These were illuminated with two rows of luminescent bulbs. A bubbler supplied carbon dioxide to the culture.

Processing of the data yielded functions $T_p = T_p(n)$ for two values of the culture's illumination.

On the basis of these experimental data, the value for $T_{p\ max}$ may be said to be $3 \cdot 10^{-5} sec^{-1}$.

The decrease in the value of T_p with increase in the culture's density may be explained by the poorer distribution of light within the suspension and provision of the cells with carbon dioxide and mineral salts. /657

The plotted curves affirm that the most favorable conditions for algae growth exist when the density of the culture is close to zero. In fact, under such conditions, each cell receives continuously the full luminous flux and does not suffer from deficiency of either carbon dioxide or mineral salts. Furthermore, the quantity of metabolites and poisons capable of inhibiting cell growth is small.

If we assume that the maximum permissible density of a culture is one where cell volume comprises half the total volume of a suspension, then since the cell diameter of Chlorella is 2 to 5 μk (average 3.5 μk), we obtain the value $n_{max} \approx 20 \cdot 10^9$ cells per cm^3.

Assuming that $q = 10^{-8} cm^3$ per cell on the average, we obtain with these values $Q_{y\ max} = 518$ liters O_2 per day per liter of suspension as the maximum specific productivity of oxygen by Chlorella.

The experimental data thus prove that in principle only one liter of Chlorella suspension is sufficient to provide a person with oxygen (560 liters per day are required). The weight and size of the biological air regeneration system would thereby satisfy the strictest requirements.

In order to attain such high productivity with a dense culture, it is necessary and sufficient to duplicate the conditions for culturing algae, chiefly the illumination conditions and supply of carbon dioxide and mineral salts, by making them conform to those that exist in diluted suspensions.

SENSORS FOR AUTOMATIC MONITORING OF THE REGULATION OF PHYSIOLOGICAL PROCESSES OF PLANTS IN CLOSED SYSTEMS

V. I. Rozhdestvenskiy and V. G. Chuchkin

In future spaceflights of long duration it is proposed to supply pas- /658
sengers with oxygen, food and water and simultaneously to regenerate the products of the vital activity of man by means of the closed ecological system (ref. 7). This system must contain a link consisting of autotropic organisms, i.e., higher and lower green plants. At the present time the application of plants appears to be the most economical and reliable method of converting the waste products of the vital activity of man (carbon dioxide, feces, urine and others) into products necessary for his existence (refs. 1 and 10). In addition to man and the autotropic organisms, the closed ecological system also contains links of physical and chemical processing of the waste products of man, so that they can be converted into a form suitable for the plants and the processing of products obtained from the plants to eliminate impurities dangerous to man (ref. 3).

The necessity of carrying out the closed ecological cycle in the hermetically sealed cabins of small size and weight places severe requirements on the amount of material in the cycle, which must be a minimum, and on the rate of its conversion, which must be rather high. It is clear that a closed system of this type will function only if all its components and links are operating properly. It appears quite unrealistic that such a complexly organized closed system could operate well and for a prolonged period of time without the control and regulation of processes which take place in its elements, including the elements containing the autotropic organisms (higher and lower plants). Other investigators have come to the same conclusions (ref. 5).

To control the rate (intensity) of the physiological processes of the /659
plants it is necessary to have instruments to indicate and record the rate of these processes, i.e., instruments to indicate the intensity of absorption and liberation of carbon dioxide, oxygen, water and individual elements of the plants' mineral food.

No such devices exist to date. The dynamics of the gaseous exchange of plants (photosynthesis and respiration), their water exchange (absorption of water and transpiration) and also the intensity of absorption of individual elements from the food solution at the present time involves a large number of measurements of the concentration of carbon dioxide or oxygen in a gaseous mixture or the concentration of individual ions in the food solution. Only after such analyses are carried out and the results of the experiments processed mathematically can the experimenter obtain information on the rate of the process.

The application of gas analyzers (for example, the infrared gas analyzer of CO_2) and automatic solution analyzers (for example, automatic flame spectrophotometer for determining K^+, Na^+ and others) sharply reduces the time necessary for obtaining the information on the flow of the physiological processes. Nevertheless, the use of even such modern instruments as the infrared gas analyzer cannot determine the intensity of photosynthesis or respiration directly in the course of the experiment. In this case the interpretation of the daily curves for the variation of carbon dioxide concentration necessary for computing the intensity of photosynthesis or respiration is rather difficult. For this reason the experimenter receives information on the dynamics of photosynthesis usually after a delay of several hours, and frequently only after a period of one to two days. Although such methods of measuring the intensity of physiological processes in spite of their shortcomings make it possible to study the physiological sides of the vital activity of plants, they are entirely unsuitable as sources of information for controlling these plant processes, particularly for systems with automatic control.

Any system which controls the rate of some process, whether it be manual or automatic, needs a device to show the current rate of the process.

Unfortunately this proposition, which is quite elementary from the standpoint of automatic control theory, is not always understood, as indicated by the work of several investigators. As an example we cite reference 2, which describes the setup for cultivating chlorella with an automatic control of its growth rate. In this setup an effort is made to use a sensor to indicate /660 the current value of the chlorella suspension density, although, as is well known, the density of the culture does not in any way reflect the rate of growth of chlorella and cannot present information on the rate of this process for the operation of an optimalizing regulator.

The design of automatic control and regulation systems for biological processes at the present time is not associated with the difficulties of constructing regulators (including optimalizing regulators) and actuating mechanisms or programming units. Such devices have been developed and some are already mass-produced. The basic difficulty is in the design of devices capable of measuring the rates of physiological processes and transforming them into electrical signals which are precisely proportional to the intensity of this process. We propose to call devices of this type intensity sensors for physiological processes. A sensor of this type may consist of a group of measurement and conversion devices which produce a combined output signal proportional to the rate of the particular process. If sensors for measuring the intensity of physiological processes are not available, it is impossible to produce a control system for the rates of these processes, especially a system for the automatic control of their rate.

In the present report an effort is made to show the potentially possible schemes for the design of sensors of this type. To make our presentation simple, we first consider the case of measuring the rate of photosynthesis and then other processes. Until recently in the Soviet Union and abroad, the intensity of the photosynthesis of whole plants as well as of individual shoots

or leaves has been measured, as a rule, with setups of two types: the plant (shoot, leaf) is placed in a hermetically sealed vessel for a certain period of time. The intensity of photosynthesis is determined from the decrease in the carbon dioxide content of the vessel; the plant (shoot, leaf) is placed in a vessel and air is pumped through this vessel. The change in the concentration at the output of the vessel is used to estimate the extent of photosynthesis (ref. 4).

In both cases the intensity of photosynthesis is computed by using the carbon dioxide balance, whose equation in the general form may be represented as

$$v_{enter}^{CO_2} = I_{\Phi} - v_{vessel}^{CO_2} + v_{exit}^{CO_2}, \quad (1)$$

where $v_{enter}^{CO_2}$ is the velocity at which the carbon dioxide enters the vessel with the plants (in ml per hour);

I_{Φ} is the intensity of plant photosynthesis (in ml/hr);

$v_{vessel}^{CO_2}$ is the rate of change of carbon dioxide content in the vessel (in ml/hr); /661

$v_{exit}^{CO_2}$ is the rate of CO_2 exit from the vessel (in ml/hr).

By transforming equation (1), we obtain

$$I_{\Phi} = v_{enter}^{CO_2} + v_{vessel}^{CO_2} - v_{exit}^{CO_2}. \quad (2)$$

In this equation

$$v_{enter}^{CO_2} = \frac{v_0 C_0}{100}; \quad (3)$$

$$v_{vessel}^{CO_2} = \frac{Q(C_1 - C_t)}{100\Delta t}; \quad (4)$$

$$v_{exit}^{CO_2} = \frac{v_t \cdot C_t}{100}, \quad (5)$$

where v_0 is the rate at which the air enters the vessel with the plant (in ml/hr);

C_0 is the CO_2 concentration in this air (in percent volume);

Q is the volume of the vessel with the plant (in ml);

C_t is the current value of carbon dioxide concentration in a vessel with a plant (air with the same CO_2 concentration exits from the chamber);

C_1 is the concentration of carbon dioxide in a vessel measured during the period Δt before its current value (in percent volume);

v_t is the rate of air flowing out of the vessel with the plant.

Substituting equations (3), (4) and (5) into equation (2) we obtain

$$I_\Phi = \frac{v_0 \cdot C_0}{100} + \frac{Q(C_1 - C_t)}{100\Delta t} - \frac{v_t \cdot C_t}{100}. \quad (6)$$

Figure 1 shows a scheme for measuring the concentration of carbon dioxide when computing the intensity of photosynthesis in a closed volume. /662 As we see from the scheme, the carbon dioxide does not enter the closed vessel and does not exit from it. Consequently,

$$\frac{v_0 \cdot C_0}{100} = \frac{v_t \cdot C_t}{100} = 0.$$

For a closed system equation (6) then takes the form

$$I_\Phi = \frac{Q(C_1 - C_t)}{100\Delta t} \quad (7)$$

If the time between the running values of the concentration in the vessel (C_t) and the concentration C_1 is constant, i.e., Δt = const, and if the volume of the vessel (Q) is the same for each setup, then

$$\frac{Q}{100\Delta t} = K_1 = \text{const}, \quad (8)$$

and equation (7) is simplified

$$I_\Phi = K_1(C_1 - C_t). \quad (9)$$

Thus, to compute the plant photosynthesis intensity in a closed volume we need to measure only quantity $C_1 - C_t$.

Figure 2 shows a scheme for measuring carbon dioxide concentration when computing the intensity of photosynthesis in a vessel with air flowing through it. As we see from the drawing, the velocity of the air which enters and leaves the vessel is the same, because there is no change in the quantity of gas inside the vessel ($v_0 = v_t$).

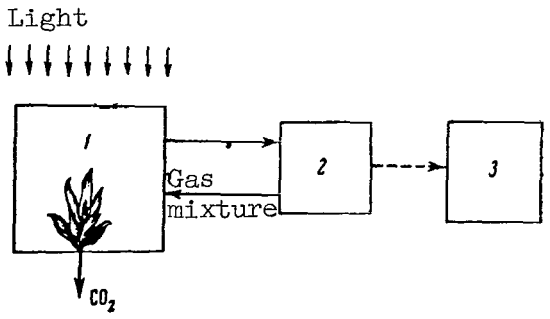

Figure 1. Measurement of CO_2 concentration for computing intensity of plant photosynthesis in closed volume. 1, Vessel with plant; 2, gas analyzer; 3, recorder.

Therefore, equation (6) may be transformed

$$I_\Phi = \frac{v_0}{100}(C_0 - C_t) + \frac{Q(C_1 - C_t)}{100\Delta t}. \tag{10}$$

If we note that the measurement of photosynthesis intensity in the air flow is made with a constant flow velocity, we have

$$v_0 = \text{const}. \tag{11}$$

Then

$$\frac{v_0}{100} = K_2 = \text{const}. \tag{12}$$

Substituting expressions (8) and (12) into equation (10) we obtain

$$I_\Phi = K_2(C_0 - C_t) + K_1(C_1 - C_t). \tag{13}$$

To compute the intensity of plant photosynthesis in a system with flowing air we must, therefore, measure two quantities: $(C_0 - C_t)$ and $(C_1 - C_t)$.

In both of the preceding cases the concentration of CO_2 in the vessel /663 with the plant is not controlled. Sometimes it is necessary to measure the plant photosynthesis taking place in air with a controlled carbon dioxide concentration.

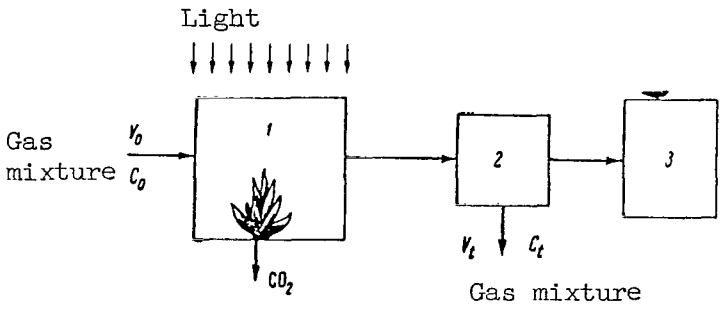

Figure 2. Measurement of CO_2 concentration for computing plant photosynthesis intensity in vessel with flowing air. Symbols same as in figure 1.

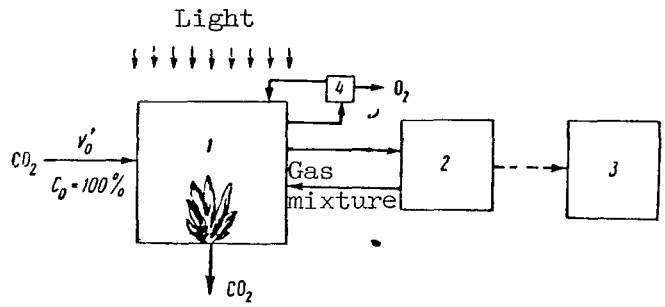

Figure 3. Measuring CO_2 concentration when computing intensity of plant photosynthesis with CO_2 concentration controlled in vessel according to fixed program. 4, Device for removing oxygen excess; other symbols same as in figure 1.

Figure 3 shows a scheme for measuring the concentration of carbon dioxide when computing the intensity of plant photosynthesis in a vessel with a controlled concentration of CO_2. The control of carbon dioxide concentration in this case is achieved by changing the rate at which pure (100 percent) carbon dioxide is supplied to the vessel. The rate at which pure carbon dioxide is supplied to the vessel is designated by v_0'.

We see from figure 3 that the carbon dioxide does not leave the vessel. Under these conditions /664

$$\frac{v_t \cdot C_t}{100} = 0. \tag{14}$$

Substituting expressions (IV) and (VIII) into equation (VI), we obtain[1]

$$I_{\Phi} = v_0' + K_1(C_1 - C_t). \tag{15}$$

Thus, to compute the intensity of plant photosynthesis in a vessel with controlled carbon dioxide concentration we must measure two quantities: v_0' and $C_0 - C_t$.

If we compare the final equations for all three cases (9), (13) and (15), we can easily see that in order to compute the intensity of photosynthesis we must know the following quantities: v_0' $(C_0 - C_t)$ and $(C_1 - C_t)$. This will show that if these quantities are measured with devices which produce electric signals proportional to the measured quantities and if such signals are added, then the total electric signal will be proportional to the intensity of plant photosynthesis. This signal can be recorded.

Let us consider individually the requirements for each of such devices.

Device 1 must transform the rate of carbon dioxide flow (v_0') into an electric signal. Therefore this device is a recording gas flowmeter.

Device 2 (differential gas analyzer) must produce an electric signal which is proportional to the difference between the input and output CO_2 concentration in air $(C_0 - C_t)$. In this case if the CO_2 concentration in the entering air is maintained at a constant level, the electric signal, proportional to the value of this CO_2 concentration in the inflowing air, will also be constant and can be furnished by an external current source.

Then the voltage, which is proportional to the difference between the input and output CO_2 concentration, will be

$$U_3 = U_1 - U_2 = K_3 C_0 - K_4 C_t,$$

where $U_1 = K_3 C_0 = \text{const}$ is the voltage corresponding to the input concentration of CO_2;

$U_2 = K_4 C_t$ is the voltage corresponding to the CO_2 concentration at the output.

[1] Translator's note: error in original text.

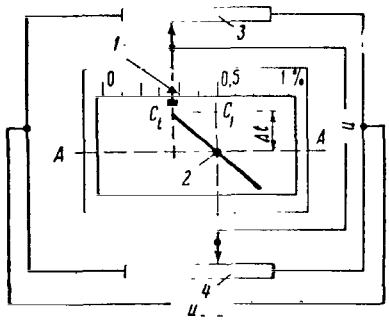

Figure 4. Diagram of functional converter. 1, Gas analyzer recorder pen; 2, photoelectric tracking head; 3, rheochord with slider mechanically connected to pen; 4, rheochord with slider connected to photoelectric tracking head.

Device 3 (functional converter) may be designed in accordance with the scheme shown in figure 4. Here the pen of the recorder (1) continuously records the current value of CO_2 concentration in the vessel (C_t), while the photoelectric tracking head (2) moves along the line A-A and continuously follows the line drawn by the pen (1). The line A-A is situated a certain distance from the pen of the recorder. This distance (in centimeters) is given by the following equation

$$l = v \cdot \Delta t,$$

where v is the speed of the recorder chart paper (in cm/hr); /665

Δ_t is the time interval between the measured concentrations C_1 and C_t.

Thus the point of intersection of the line (A-A) with the line on the diagram corresponds to the value of C_1.

Rheochords (3) and (4) are mechanically connected to the pen of the recorder and the photoelectric tracking head. At the bridge output composed of these two rheochords, an intensity proportional to the concentration differences C_1 and C_t results:

$$U_4 = K_5 (C_1 - C_t).$$

Thus, for all these cases considered here the measurement of photosynthesis intensity (in a closed volume, in an air flow and with regulated CO_2 concentration in a closed volume) may be accomplished by means of these three devices.

In the first and second case the sensors for the intensity of photosynthesis consist of a gas analyzer in a functional converter, while in the third case they consist of a gas analyzer, a functional converter and a gas flowmeter.

Let us consider the possibility of designing similar sensors for measuring other physiological processes of plants. We shall consider the sensors for measuring the intensity of absorption by the roots of water and other elements of the food solution as sensors for absorbing water and sensors for absorbing potassium ions.

Figure 5 shows the diagram for measuring the concentrations of K^+ /666 ion in the food solution during the computation of the intensity of its absorption by the roots of the plant, when the concentration of this element is controlled in the food solution. The control of K^+ concentration is achieved by varying the rate (v_0) at which the concentrated (C_0) solution of K^+ is supplied.

By transforming the equation for the potassium balance in the same manner as equation (1) for carbon dioxide, we have

$$I_k = K_1 v_0 - K_2 v_t C_t + K_3 (C_1 - C_t), \tag{16}$$

where I_k is the intensity of potassium ion absorption (in mg/hr);

v_0 is the rate at which a concentrated solution of potassium is supplied;

v_t is the rate at which the feeding solution leaves the vessel with the plant;

C_t is the concentration of K^+ in the feeding solution, measured by the flame spectrophotometer;

C_1 is the concentration of K^+ in the feeding solution which existed during the time Δt before its current value (C_t);

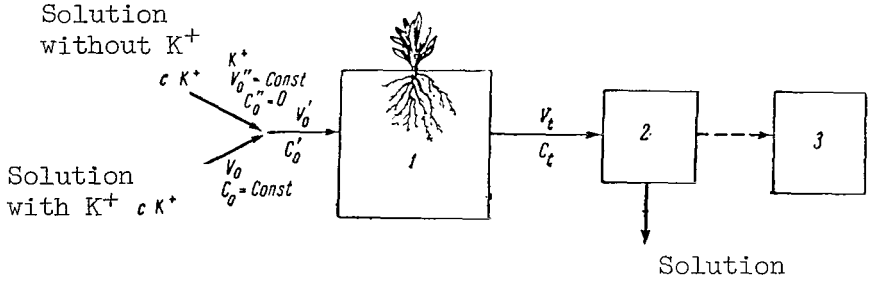

Figure 5. Measurement of K^+ ion concentration in feed solution when computing intensity of this ion absorption by plant. 1, Vessel with plant root system; 2, flame spectrophotometer; 3, recorder for recording variation in K^+ concentration of solution.

K_1, K_2 and K_3 are constant coefficients because $K_1 = C_0/100$, where C_0 is the potassium concentration in the concentrated solution

$$K_2 = 0{,}01 \text{ and } K_3 = \frac{Q}{100\Delta t},$$

where Q is the volume of the feeding solution in the vessel with the root system of the plant, which is maintained at a fixed value because excess of the solution is poured off. The quantity Δt, as in the case described, is selected to be constant.

Thus, in order to record the intensity of K^+ absorption by the plant automatically, it is necessary to measure the following quantities: v_0, v_t, C_t and $(C_1 - C_t)$.

The rate of flow of the solutions (v_0 and v_t) was measured by means of flowmeters; in case of small and ultrasmall rates (up to 0.5 ml/hr), it is convenient to use a device described in the literature (ref. 6).

The current values of the K^+ ion concentration are measured by the flame spectrophotometer with an automatic compensation for sensitivity (ref. 8). /667

The quantity $(C_1 - C_t)$ may be measured by the functional converter described above.

If we note that the absorption of minerals from the solution has practically no effect on its volume, the intensity of water absorption will be equal to

$$I_{H_2O} = v_0' - v_t, \tag{17}$$

where v_0' is the rate at which the solution is fed into the vessel with the plant (in ml/hr); /668

v_t is the rate at which the feeding solution flows out of the vessel with the plant (in ml/hr).

Since $v_0' = v_0 + v''$, we have $I_{H_2O} = v_0 + v_0'' - v_t$. The quantities v_0 and v_t are measured by the flowmeters, while the quantity v_0'' is constant and may be assigned by an electric signal generated to a definite scale.

Figure 6 shows the diagram for the automatic control of potassium concentration in the feeding solution, with a simultaneous recording of the absorption

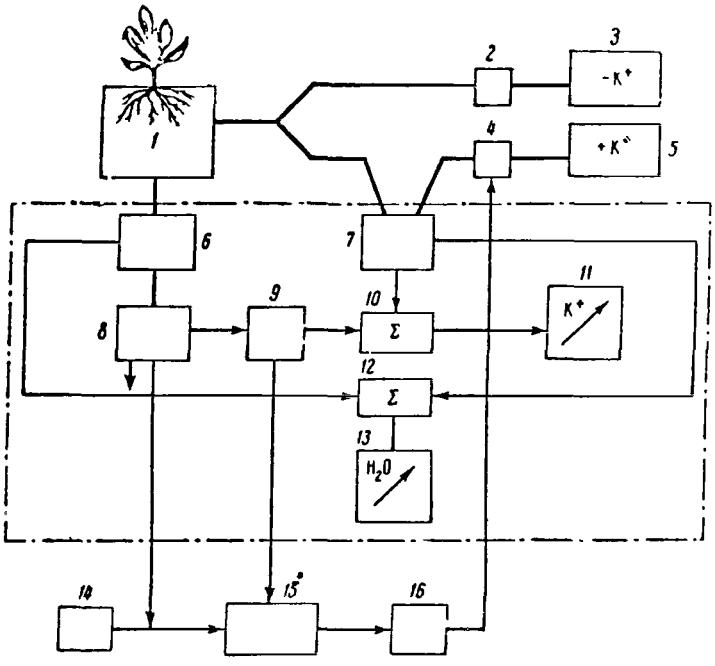

Figure 6. Automatic control of potassium concentration in feeding solution with simultaneous recording of intensity with which this ion and water are absorbed by plant. 1, Vessel with plant root system; 2, 4, pumps for feeding solution; 3, vessel with solution without K^+: 5, vessel containing a solution with K^+; 6, 7, liquid flowmeter; 8, flame spectrophotometer; 9, functional converter; 10, 12, adders; 11, recorder for K^+ absorption; 13, recorder for water absorption; 14, programming unit for potassium; 15, regulator; 16, actuating mechanism.

intensity of this ion and of water by the plant roots. The broken line isolates the devices contained in the sensor for measuring the absorption intensity of potassium ion and of water.

The setup operates in the following manner: the current value of potassium concentration in the vessel with the plant (C_t) is measured by the flame spectrophotometer (8). The amplified electric signal, proportional to this concentration, is compared with the electric signal produced by the programming unit (14) for the concentration of potassium in the solution. The difference between these two electric signals is fed to the regulator (15). A signal from the functional converter (9) is also fed to this device. The actuating mechanism (16), controlled by the regulator, reacts on the rate of concentrated potassium solution flow into the feeding solution until the concentration /669 of this element in the feeding solution contained in the vessel with the plant equals the programmed value.

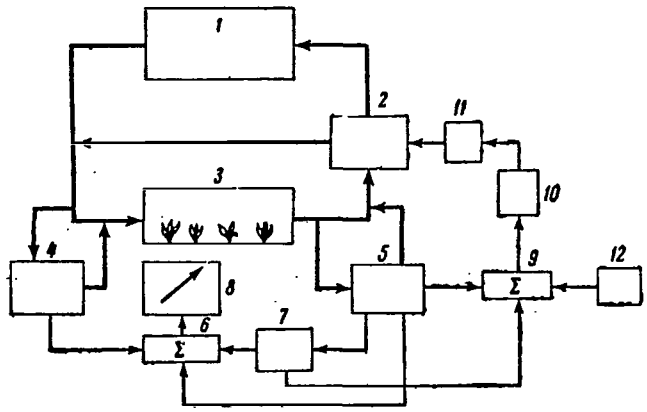

Figure 7. Automatic control of photosynthesis intensity in closed ecological system. 1, Chamber with man; 2, CO_2 concentrator; 3, greenhouse; 4, 5, gas analyzers; 6, 9, adders; 7, functional converter; 8, recorder for photosynthesis intensity; 10, regulator; 11, actuating mechanism; 12, programming unit.

Figure 7 shows a diagram of one of the possible versions for the automatic control of photosynthesis intensity in a closed ecological system for future spacecraft, where the intensity of photosynthesis is simultaneously recorded.

The closed gas system differs from the one already considered only in that the unit for absorbing oxygen and the CO_2 source, shown in figure 2, are replaced in this case by a chamber (cabin) with man (1) and a CO_2 concentrator (2).

Since the vessel with the plant is connected to the closed gas loop, the rate at which air and CO_2 enter the chamber is equal to the rate at which it leaves the chamber with the plant. Therefore, the quantity of CO_2 supplied to the vessel with the plant is proportional to its concentration at the input and is measured by the gas analyzer (4). As we see from figure 7, the air enriched with carbon dioxide is blown through the vessel with the plant. The CO_2 concentration in this chamber is automatically controlled in accordance with a program assigned by the programming unit (12). At the same time the recorder (8) records the current values of photosynthesis intensity associated with plants placed in the chamber.

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SPECIAL FEATURES OF PLANT FEEDING UNDER CONDITIONS OF AEROPONIC CULTIVATION FOR A CLOSED SYSTEM

I. V. Tsvetkova, Yu. I. Shaydarov and V. M. Abramova

The nutrition of higher plants in a closed ecological system should /670
provide for mineralized human metabolites. One way of mineralizing them is by the biological method, whereby the organic wastes are mineralized in a biological filter by microorganisms. The use of mineralized human metabolites for feeding higher plants poses the danger of salinizing the nutrient solution with sodium chloride, because about 300 mg/liter chloride is added to that solution daily with the mineralized metabolites, about as much as nitrogen. In view of the different rates of nitrogen and sodium chloride absorption by plants, chlorine and sodium ions will steadily accumulate in the nutrient solution. Their concentration may become toxic for the plants under cultivation. Furthermore, human metabolites contain physiologically active substances whose molecular fragments may remain in the solution after the mineralization, and produce an inhibiting or stimulating effect on the plants.

The purpose of this investigation, therefore, was to study the absorption of certain elements of the mineral plant food from solutions consisting of mineralized human metabolites, as well as the absorption of chlorine and sodium ions by the increasing concentration of chlorides in the nutrient solution.

Our object of investigation was Chinese cabbage (from Hibin seeds). The seedlings were cultivated in sand. When the plants developed three regular leaves, they were taken to be raised by the air culture method. The latter /671
is one of the prospective plant-growing methods (refs. 1, 2, 5, 6, 10, 12, 13 and 15).

Under this principle a nutrient solution is periodically and automatically sprayed in the area of the plant's root system (30 sec every 20 min), which is kept in a closed air space saturated with water vapors. The advantage of this method is the absence of a gravimetric substrate which adsorbs some of the plant food elements on its surface, as well as the small volume of the nutrient solution, which facilitates a highly accurate determination of the changing concentration of the nutrient mixture components. The absence of a substrate and the small volume of the nutrient solution reduce the weight of the entire installation and make it a promising device for use in space investigations.

The following system was used for the experiments: (1) the plants were cultivated on a mineral mixture without salinization (control); (2) the plants were raised on a mineral mixture with an increasing concentration of sodium chloride (from 0.02 to 2.0 percent by chlorine-ion count), and (3) the plants were raised on solutions of human waste products, adjusted for shortage of mineral nutrients.

In the course of the investigations into the utilization of mineralized human metabolites for plant food, it was found that their chemical composition could not supply the normal nutrition for the plants, and the addition of mineral salts therefore became necessary. To approach the level of standard nutrient mixtures, we calculated the adjustment for the shortage of the mineral nutrition elements (nitrogen, phosphorus and potassium) in the solution of the mineralized human metabolites every 10-12 days. A mineral mixture with tap water was used as a control for this mixture in order to ascertain the specificity of action of the mineralized solution. Based on mineralized human metabolites the nutrient elements of this solution were almost equal to those of the standard mineral solutions used for cultivating higher plants.

In the study of the absorption of the basic mineral nutrients, the total nitrogen was determined by the Kjeldahl method, ammoniun nitrogen by the Nessler reagent, the nitrate nitrogen by the Greenwald-Lewman method, the nitrite nitrogen by the Griess method, the urea nitrate by the Ehrlich reagent, the phosphorus by the Denigés method, the sodium and potassium by the method of flame photometry, and the chlorine by the usual titration with silver nitrate.

The absorption of the elements in the nutrient solution was determined by the difference in their content. The water used in the process was measured daily, correct to 10 milliliters, and the solution pH was maintained at 5.6 throughout the entire experiment. The salinization of sodium chloride was carried out gradually every 4-5 days. It was done in an air-conditioned chamber with natural light (temperature 20-25°C, relative humidity 70-80 percent) /671 at the artificial climate station of the Plant Physiology K. A. Timiryazeva Institute of the USSR Academy of Sciences.

The analyses based on the absorption of the basic mineral nutrient by the plants cultivated on solutions including mineralized human metabolites revealed that mineralized human metabolites can be used for the cultivation of higher plants, with appropriate adjustments for the shortage of mineral nutrient elements.

Far from producing any specific effect, the mineralized human metabolites, with appropriate adjustments, actually produced a stimulating effect, manifested in an intensified absorption of the basic mineral nutrients and the emergence of a productive biological mass (tables 1 and 2).

It was further ascertained that plants can be cultivated for 30-40 days in an air culture medium and a permanent nutrient solution to which salts are added as they are absorbed. The plants eventually die, even though the so- /673 lution remains optimal. Special tests, however, revealed the nutrient solution to be toxic, probably because of the accumulation of metabolites

produced by the root secretions in the cultivation of plants, as noted by a number of authors (refs. 3, 7 and 11). However, some researchers hold the opposite view (ref. 4), as the study of the root secretion involved the cultivation of plants by the sand and water culture media, in which the metabolic products cannot accumulate or produce a toxic effect because of the large volume of solution per plant or the presence of a substrate.

TABLE 1. ABSORPTION OF BASIC MINERAL NUTRIENTS BY 10 CABBAGE PLANTS (AGED 20-32 DAYS) IN COURSE OF 12 DAYS (IN mg).

Type of test	Total nitrogen	NH_4	NO_3	$CO(NH_2)_2$	P	K	Na	Cl
Mineral mixture	1,019	626	220	175	260	912	421	1,500
Mineralized metabolic products	1,194	665	354	175	303	1,380	484	1,700

TABLE 2. CABBAGE CROP STRUCTURE.

Type of test	Plant height, cm	Number of leaves	Live wt of above-ground biomass of 10 plants, gr	Dry wt of above-ground biomass of 10 plants, gr
Mineral mixture	24.9	8.4	319	37.0
Mineralized metabolic products	31.0	11.4	424	48.1

TABLE 3. ABSORPTION OF MAJOR MINERAL NUTRIENTS BY 10 PLANTS (AGED 33-45 DAYS) IN 12 DAYS (IN mg).

Type of text	N			P	K	Na	Cl
	Total	NH_4	NO_3				
Mineral mixture	94*	706*	147*	272	24.7	321	471
Mineralized metabolic products	286*	700*	213*	340	35.0	352	654
Mineral mixture with a 0.1-0.3 percent chlor-ion salinization	1,233	490	50	600	1,206	1,196	8,675

*Secreted.

The toxic effect of the solution was a sharp disturbance of the absorptive functions of the root systems. An analysis of the solution revealed a reduced absorption of potassium, phosphorus, chlorine and sodium, while the nitrogen (produced mostly by ammiac forms) was secreted by the root systems into the nutrient solution (table 3).

The toxic substances resulting from the root metabolism probably affect not only the absorptive, but also the synthetic functions of the root systems, which apparently leads to a partial albumin proteolysis and ammonium secretion (table 3). Such phenomena have been observed as a result of disturbed synthetic activity of the root systems in dry air conditions (ref. 9).

Consequently, the cultivation of plants by the air culture method with a small amount of permanent nutrient solution, to which only nutritive substances and water are added, will result in accumulation of toxic substances in the solution and the eventual destruction of the plants. But no such phenomenon was observed when the solution was salinized with sodium chloride to 0.1 per- /674
cent; the absorption of the major mineral nutrients continued at the previous level (table 3).

Obviously, the presence of sodium chloride in the nutrient solution prevents the accumulation of the soluble toxic products of root metabolism or the metabolic products of the microflora. The causes and mechanism of this phenomenon call for further investigation. In view of the fact that the plants cultivated on a mineral nutrient solution died between the 45th and 50th day of the experiment, the further investigation was based only on the type of experiment involving gradual salinization. The purpose of these investigations was to study the absorption of the various concentrations of sodium and chlorine ions from the nutrient solution by the plants, and to determine the toxicity threshold by the sodium chloride. The mineral nutrient solution was salinated by the chlorion method from 0.1 to 2 percent. As proved by the analysis results, the absorption of chlorine and sodium ions by the plants increases sharply with the increasing concentration of sodium chloride in the solution (table 4).

TABLE 4. ABSORPTION OF VARIOUS CONCENTRATIONS OF CHLORINE AND SODIUM IONS IN NUTRIENT SOLUTION.

Chlorine		Sodium	
Initial concentration, mg/l	Absorption by 10 plants in 24 hr, mg	Initial concentration, mg/l	Absorption by 10 plants in 24 hr, mg
250	142	100	48
1,000-3,000	723	650-1,340	100
10,000	8,300	2,600-3,880	276
13,000-20,000	8,167	6,500	601

We were unable to reach the chlor-ion toxicity threshold in our experiments, as by the time the salinization reached 2 percent, the plants had completed their vegetation and the experiment was discontinued. The salinization degree of the nutrient solution did not affect the growth, development or yield of the plants, as the data in table 5 indicate.

TABLE 5. EFFECT OF VARIOUS DEGREES OF SALINIZATION OF NUTRIENT SOLUTION ON CABBAGE CROP STRUCTURE.

Type of test	Plant height, cm	No. of leaves	Weight of above-ground biological mass of 10 plants, g	
			Wet	Dry
Mineral mixture	24.9	8.4	319.0	37.0
Mineral mixture with 0.2-0.6 percent chlor-ion	26.0	9.5	505.0	38.0

The data on the plant yield are indicative of an increase in the gross live weight of the productive biological mass in the variant test involving the salinization of the nutrient solution, while the dry weight was practically indifferent from that of the control. Manifested in this, apparently, is the specificity of the plant's reaction to the presence of chlor-ions in the nutrient solution, a specificity expressed by an increasing hydration, or succulence of the cells by a chloride salinization (ref. 8). /675

Plants cultivated in an air culture are characterized by a higher degree of salt endurance than plants raised on various substrates (ref. 14). This is apparently due to the higher resistance of the roots and plants as a whole, facilitated by better aeration and water supply of the root systems. This is probably why the plants raised in an air culture are capable of enduring a fairly high concentration of sodium chloride in a nutrient solution.

The experimental results revealed that even a considerable increase of the chlorine concentration in an air culture does not produce a toxic effect on the plants. Consequently, the introduction of chlorides with the mineralized human metabolic products cannot increase the salinization to a plant-destroying degree because of the high level of salt endurance in an air culture.

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CONDITIONS OF CARBON NUTRITION OF CHLORELLA IN INTENSIVE CULTURES

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Carbon nutrition of chlorella was made possible by carbon dioxide /676
dissolved in the culture medium; chlorella can assimilate bicarbonate of carbon only in case of a carbon dioxide shortage, and after some adaptation (refs. 1 and 7).

In this connection, the carbon dioxide curves of the chlorella photosynthesis, found in the literature, are presented as concentration functions of molecular carbon dioxide.

The factual data obtained in this field by various authors are contradictory. As shown in references 3 and 5-7, they obtained photosynthetic saturation of 0.05 percent chlorella concentration in air. At the same time, the saturation concentration of carbon dioxide was estimated at 0.5 percent (ref. 2); further increase in the carbon dioxide content in air to 5 percent did not reveal any reduction in photosynthesis (ref. 2), while an increase in concentration to 1 percent was found to inhibit photosynthesis (refs. 5 and 6). This carbon dioxide concentration was observed to inhibit cell division, and concentration above 5 percent resulted in complete cessation of division (ref. 4).

The wide range of saturated carbon dioxide concentrations, referred to by various authors, prompts the assumption that the carbon dioxide saturation threshold of photosynthesis, all other optimal cultivation conditions being equal, is not always typical of the physiological characteristics of chlorella cells alone. It is quite probable that it also reflects various cultivation conditions responsible for different rates of carbon dioxide consumption by cells.

Our experiments also revealed a variety of carbon dioxide saturating concentrations. Thus, in the cultivation of chlorella in suspensions of 100-150 million cells per milliliter, saturation of photosynthesis was observed at 0.2 percent concentration of carbon dioxide in air. An optimal culture productivity was achieved at $\sim$ 1.5 percent concentration of carbon dioxide in air by increasing the suspension density to 500-600 million cells per milliliter. /677

Actually a change from low suspension densities to an intensive culture with a view to achieving maximum suspension productivity revealed increase of saturating carbon dioxide concentrations in air to 1.4-1.6 percent. These

carbon dioxide concentrations have now been adopted by a number of laboratories experimenting with intensive chlorella culture.

Earlier we have shown that the effective method of increasing the specific productivity of the chlorella suspension is an increase in the suspension density and the preservation of the optimal conditions for each cell (ref. 8).

It appears that in addition to the distribution of light in the suspension and the maintenance of an optimal composition of the medium it is just as necessary to create optimal conditions for carbon nutrition, which, by and large, probably means maintenance of a certain potential strength of the dissolved carbon dioxide in the medium washing the cells.

It is natural to assume that maintenance of the necessary carbon dioxide concentration in the cell zone, in keeping with the intensity of its consumption, will require (all other conditions being equal) a varying concentration of carbon dioxide in air. This project was undertaken with a view to verifying that assumption.

The experiments were carried out in an airtight system with an enclosed air space. The capacity of the gas system was 9 liters, which made it possible to use the same culture for three consecutive experiments without any substantial changes in the suspension density or the medium. An algae culture, cultivated beforehand, was centrifuged for 15 min at 3,000-4,000 rpm and suspended again in a fresh Tamiy nutrient medium. The density was brought up to the level required by the experiment, and the suspension placed in a reactor. After some adaptation, the necessary carbon dioxide concentration was produced in the system in the course of 1-2 hours, and the experiment began. The mixture of air and carbon dioxide was forced in by a diaphragm pump at a rate of 3-4 liters per minute. Based on the film method of cultivation, the reactor provided good conditions for the gas exchange between the open suspension surface and the air. The photosynthetic rate was defined as a function of the diminishing content of carbon dioxide in the closed system by means of a continuous-operation infrared gas analyzer (UAV-1).

The measurements were made within the range of carbon dioxide concentrations from 12-13 percent and lower to complete cessation of visible photosynthesis. The pH of the medium fluctuated from 5.5 early in the experiment to 6.0 at the end.

A thermophilic chlorella strain with a temperature optimum of 40-41° was used in the experiments.

The suspensions used in the investigations had a density of /678

0.5-0.6 · 10^9, 3-4 · 10^9 and 8-10 · 10^9 cells per milliliter. The data obtained from all experiments were fully monotypic. Some of them are cited as examples in figures 1, 2 and 3.

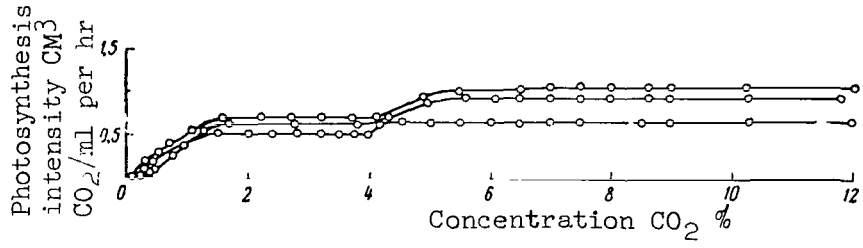

Figure 1. Relationship between photosynthesis intensity in chlorella culture medium and carbon dioxide concentration in air, with suspension density amounting to 5-6 • 10^8 cells/milliliter.

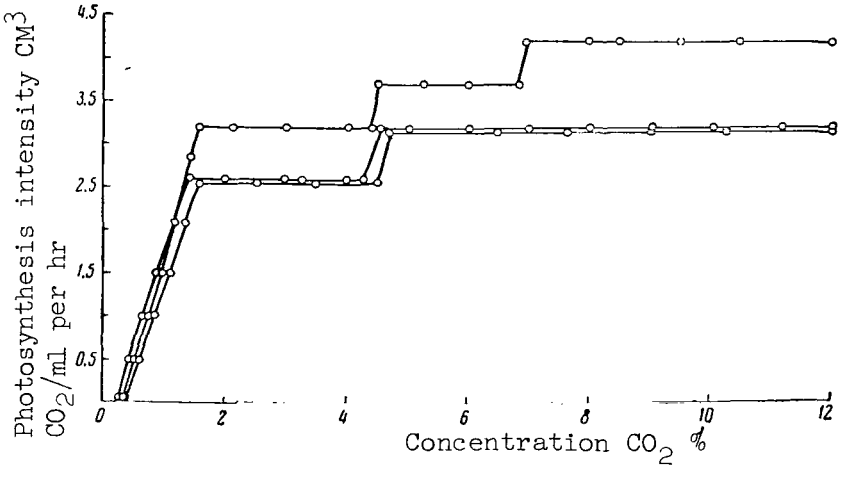

Figure 2. Relationship between photosynthesis intensity in chlorella culture medium and carbon dioxide concentration in air, with suspension density amounting to 4-4.9 • 10^9 cells/milliliter.

As indicated in the figures, the first extension of the curves to the plateau, regardless of the suspension density, was observed at 1.5-1.8 percent carbon dioxide concentrations in air. A further increase in the carbon dioxide concentration in air to 12 percent has led to an intermittent increase in productivity at very definite concentrations. The second outlet to the plateau was observed at 4.5-5.5 percent carbon dioxide concentrations in air. The third outlet of curves to the plateau, characteristic of a density of 10 • 10^9 cells per 1 milliliter (fig. 3) and, in cases of most intensive photosynthesis, also of 4 • 10^9 cells per 1 milliliter (fig. 2, upper curve), was observed with the 7.0-7.5 percent range of concentrations. A further increase /679

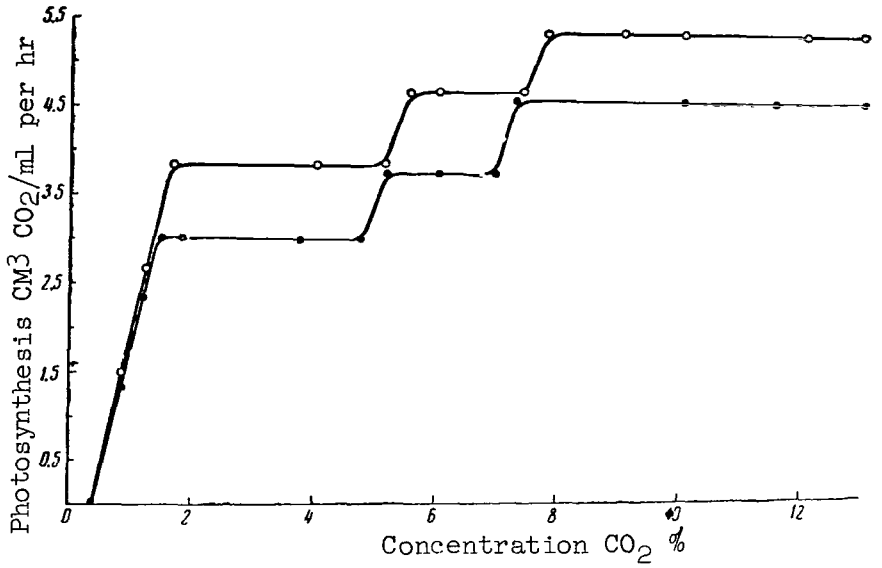

Figure 3. Relationship between photosynthesis intensity in chlorella culture medium and carbon dioxide concentration in air, with suspension density amounting to $10 \cdot 10^9$ cells/milliliter.

of carbon dioxide in the air to 8-12 percent failed to produce an increase in culture productivity; there was no inhibition of photosynthesis.

Thus, the saturation concentrations of carbon dioxide for a high-density intensive chlorella culture were found to be higher than those obtained earlier. The multistep shape of the resulting curves is probably due to various phenomena limiting the rate of oxygen supply to the fixation spot in the cell.

Actually, all changes of gaseous carbon dioxide to a solution may prove to be such limiting factors: diffusion of carbon dioxide to the cells and through the cell membrane, its movement to the fixation spot as well as the rate of carboxylation.

It may be assumed that each of these processes depends on the carbon dioxide concentration, which may be raised by increasing its concentration in air. In this connection, each of the jumps in the suspension productivity shown in figures 1, 2 and 3 may be interpreted as resulting from the local "saturation threshold" in any of these carbon dioxide transfer links, which tends to limit the overall rate of this process and also, therefore, /680 the rate of photosynthesis recorded in the experiment, when the content of carbon dioxide is low.

The rate of carbon dioxide consumption by the cells has a direct affect on the partial carbon dioxide pressure in the air required to maintain its optimal concentration in all stages of the movement. This probably explains the

presence of a third outlet to the plateau in a culture with a high photosynthetic intensity (fig. 2, upper curve), and its absence at a lower rate of carbon dioxide consumption (fig. 2, bottom curve).

Thus, the realization of the optimal cell cultivation conditions, conducive to a higher photosynthesis intensity in the cell, increases the gradient of carbon dioxide concentration between the pericellular layer of the medium and the surface of its contact with the gaseous phase. This increase in the gradient has a positive significance, because it creates the conditions for faster change of gaseous carbon dioxide to a solution and its diffusion to the cells. However, there is no advantage to increasing the carbon dioxide gradient in the solution merely by reducing its level in the pericellular layer, because its partial pressure in that zone may drop below the magnitudes required for an adequate rate of carbon dioxide consumption by the cell.

This physiological minimum carbon dioxide concentration in the pericellular zone of the nutrient medium may, in the first approximation, be adopted the way it appears during the threshold concentration in the low-density suspensions, where the carbon dioxide gradient inside the nutrient medium may be disregarded.

An increase in the suspension density, reducing the volume of the medium per cell, is also of considerable importance for the carbon dioxide nutrition of algae.

A decrease in the specific volume of the medium is conducive to a corresponding reduction in the available quantity of dissolved carbon dioxide in the area of each cell, and it is also inevitably interlinked with the overall surface of this individual "nutrition" sphere, the inflow channel of new carbon dioxide molecules. The incipient reduction in the total number of dissolved molecules in the medium and the decreasing specific surface of the exchange with the source of carbon dioxide supply are also conducive to a lower concentration of carbon dioxide in the pericellular zone. Naturally, in this case there is the possibility of the partial carbon dioxide pressure in the medium dropping to a level where the necessary rate of its inflow to the cell can no longer be assured, despite the "saturation" concentration of it in a gaseous phase.

It should be clear, in view of these considerations, that the absolute prerequisite for the inflow of carbon dioxide to the cell at the required /681 rate is not its concentration in the gaseous phase, but a certain tension of the dissolved carbon dioxide in the cell zone. The carbon dioxide content in the air is merely a precondition for the maintenance of such a gradient in its medium, whereby the lower level of the carbon dioxide partial pressure does not drop below the physiologically required magnitudes.

However, the carbon dioxide concentration in air is not by far the only condition for maintenance of the necessary gradient in its solution. Such conditions are presented by all other factors affecting the gas exchange rate

between the air and liquid phases: the phase contact area, the rate of molecular diffusion in the nutrient medium and the mobility of the medium itself.

The most controlled of these factors, important for intensive cultivation, is the suspension-air contact area, an increase of which increases the quantity of dissolved carbon dioxide at a given partial pressure.

The specific contact area between the suspension and air (the ratio of the phase separation area to the suspension volume) should therefore be considered one of the essential design characteristics of photosynthetic reactors. This is quite important for the construction of closed gas-exchange man-chlorella systems, where the carbon dioxide concentration in air can hardly exceed the permissible magnitude for human beings, and where special devices are required to increase its concentration, thus reducing the economic efficiency of the system.

With such a complex relationship between the carbon dioxide supply to the cells and various factors, it would be erroneous to consider the saturation concentration of carbon dioxide in air a constant with a separate value. This magnitude may vary under different concrete cultivation conditions, and its definition will always be of limited importance for the given conditions.

Thus the completed investigations show that the use of high-density chlorella suspensions and the maintenance of an intensive culture medium, when the productivity of 1 liter of suspension exceeds 100-130 liters of carbon dioxide in 24 hours, call for creation of conditions for rapid and continuous replenishment of the dissolved carbon dioxide in the photosynthesizing cell zone. Such conditions can be achieved not only by increasing the carbon dioxide concentration in air, but also by a number of other measures designed to facilitate the dissolution of carbon dioxide and the delivery of the dissolved gas to the cells (an increase in the gas-exchange surface, a decrease in the thickness of the suspension layer and effective stirring).

This indicates that identical conditions for the carbon nutrition /682
of cells can be achieved with various carbon dioxide concentrations in air, and vice versa: the same concentration of carbon dioxide in a gaseous phase can, under different conditions, produce different levels of cell nutrition.

The data obtained in the investigation of carbon nutrition in algae culture media under various conditions of cultivation can, therefore, be compared only on the basis of available data on the concentration of carbon dioxide dissolved in a nutrient medium at certain pH values and other permanent kinetic conditions.

A fuller solution of these questions requires special investigation with more thorough analysis of the physicochemical conditions governing the solution and delivery of carbon dioxide in the nutrient medium, as well as its diffusion through the cell membrane and to the fixation spot in the cell.

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DENSE CONTINUOUS CULTIVATION OF CHLORELLA UNDER VARIOUS ILLUMINATION CONDITIONS

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The purpose of this study was to determine the optimal illumination /683 of chlorella algae suspensions with a high concentration of cells in a continuous intensive cultivation. The tests were made with suspensions of a thermophilic strain of Chlorella vulgaris algae, which have a density of $2 \cdot 10^9$, $3 \cdot 10^9$ and $4 \cdot 10^9$ cells per 1 milliliter, or 20, 30 and 40 g of dry biological mass per 1 liter of suspension, respectively. The algae were cultivated in a flat cultivator with an effective capacity of 1.4 liters, a dark space of 0.25 liter and a total effective surface of 0.6 m^2.

In the course of the experiment, the suspension, temperature (35.5 ± 0.7°C), pH (7.35 ± 0.4), layer thickness (5 mm), air flow through the cell suspension, CO_2 content in air (5 percent) and the composition of the nutrient medium were maintained at certain levels facilitating a relatively intensive biosynthesis. The scavenging air with a high CO_2 content used for blowing through the culture stirs up the suspension quite well. It should be pointed out that the complications arising in the suspension in connection with the illumination of individual cells consist in the periodic changes in intensity and spectral composition of the light illuminating them.

The special investigations carried out for the purpose of studying the general patterns of light attenuation and diffusion in suspensions of unicellular algae revealed that the attenuation of monochromatic rays and white light in the first section of the optical path is governed by a law similar to the exponential one. The absorption coefficients of the monochromatic rays increase with the increasing penetration of the radiation as a result of the repeated scattering and lengthening of the optical path, while the integral absorption coefficients of the white light decrease as a result of the /684 uneven attenuation of the radiation on various wavelengths. About 90 percent of all photosynthetically active radiation (PAR) is absorbed on a 0.5 mm optical path in a suspension with a density of 30 g of dry weight of the biological mass per 1 liter, and the radiation completing that path actually consists of a green light. Stirring the culture tends to alternate the light and dark phases of each cell.

The changing illumination period and the correlation between the shady and light phases of the individual cells, as well as their average illumination, depend on the intensity and spectral composition of the incoming radiation, the illumination of the culture, its density, the thickness of the suspension layer and the stirring bubbling intensity.

In our experiments a 5 mm thick layer of algae suspension was illuminated in a culture medium evenly on both sides by DRL-1000 and ND-2 gas-discharge tubes, which produce relatively favorable radiation for cell photosynthesis. In all experiments involving culture media of various densities the illumination was changed in a stepwise manner (a total of 6 illumination levels ranging from 0.260 to 1.202 cal/cm^2/min). The illumination usually changed from a minimum to a maximum, and vice versa. Each step lasted 4-5 hours. The fluctuation of the illumination from any preselected level was not more than ±4 percent. The continuous tests lasted six days.

A thermophilic Chlorella vulgaris strain, kept for four months under continuous cultivation, was used for these tests.

The major index used for evaluating the effect of various degrees of radiation in the algae growth was the productivity, expressed in accretion of the biological mass in grams of dry substance per liter of suspension in 24 hours.

A comparison of the average culture productivities at each illumination level produces a clearer picture of the relationship between the intensity of biosynthesis and the illumination level of the suspension. A graphic representation of that relationship with regard to these three culture densities is found in figures 1a, b and c.

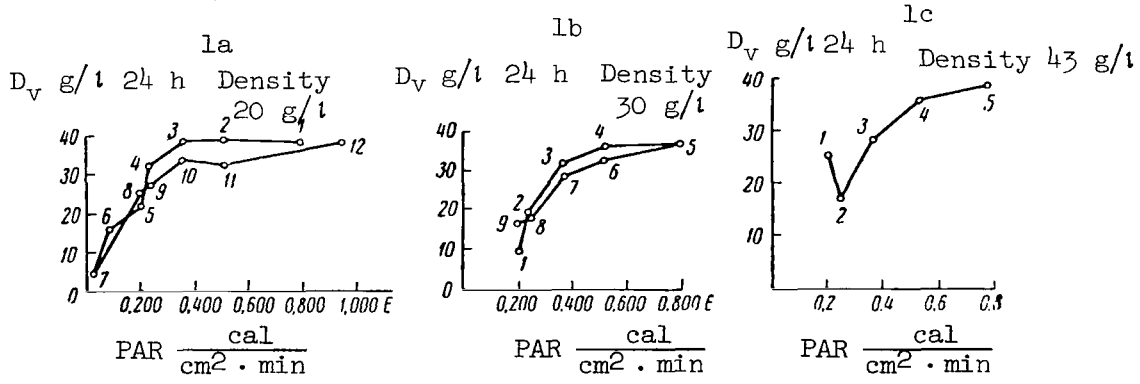

Figure 1. Relationship between average productivity of culture medium and illumination of suspensions with densities of 20, 30 and 40 g of dry weight of biological mass per milliliter, respectively, D = density.

These graphs show that an increase in illumination is at first followed by a rapid productivity growth of the culture medium with the three densities under investigation. Later, when a certain intensity of illumination is

reached, this growth becomes insignificant, which means that further increase in illumination is no longer conducive to a marked accretion of productivity. In some cases increase in illumination far beyond that level actually resulted in diminishing productivity. Distinctly different illumination levels facilitating maximum productivity were achieved in the cultivation conditions under consideration. This level rises from 0.361 to 0.791 cal/cm^2/min PAR with increasing culture density. In this case the productivity of the three different densities was found to be almost the same (from 36-38 g of dry weight per liter of suspension in 24 hours, or 117 ± 12.4 g of dry substance per 1 m^2 of effective surface in 24 hours). /685

It should be pointed out that an intense culture and density illumination, producing more favorable conditions for bacteria growth, should not be permitted in cases of lengthy cultivation. In suspensions with densities of 20, 30 and 45 g/liter, the bulk of the bacteria amounted to 2, 6 and 8 percent of the total biological mass, respectively.

The approximately similar maximum productivities can be explained by the fact that a 5 mm thick culture with high concentration cells is, from an optical point of view, very dense. Thus, an 0.5 mm layer with a density of 30 g/liter absorbs up to 90 percent of radiation, that is, taking account of the fact that a bilateral illumination of the culture medium illuminates only one fifth of the cells at a time. With a density of 43 g/liter, the portion of the culture illuminated at one particular time is still smaller. It should be pointed out that thorough mixing will produce the conditions whereby all cells will be found at various depths with equal probability, and their illumination will change periodically in the same way in point of magnitude and spectral composition. It appears that the average illumination of the cells in a fixed-thickness layer is a function of the surface illumination and density of the culture (fig. 2); it sharply diminishes with an increase in the latter. In

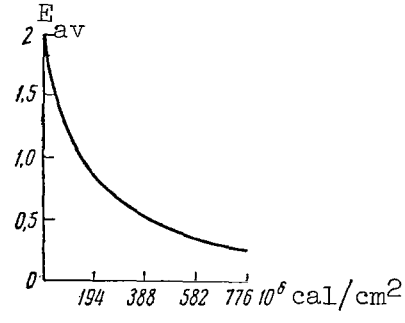

Figure 2. Relationship between average E_{av} illumination of cells and nl cells/cm^2 magnitude of chlorella algae illuminated on both sides by DRL tubes (n--concentration of cells in cm^3, l--layer thickness).

this connection, the productivity of the individual cells also diminishes with increasing density. Thus, in the case of a 20 g/liter density, the cultivation period during which the suspension becomes equal to the effective cell suspension is 10 hours, while in the case of a 43 g/liter density it is 28 /686 hours, which means that in the latter case the intensity of the cell biosynthesis is almost three times lower.

By reducing the layer thickness we obtained a considerably higher productivity per unit of volume (80-90 g/liter in 24 hours), but this did not increase the overall productivity of the culture.

An increase in the overall productivity by the use of intensive light and high cell densities can be achieved only by increasing the surface exposed to the light in a culture of the same size and design. One way of doing it is to complicate the shape of the illuminated surface (by wedges and bends). The adequately illuminated share of the cells will increase with the increasing surface, and the culture as a whole will work more productively; furthermore, an increase in the surface of the same culture volume may produce the most favorable mean illumination values for the individual cells at a given light of high intensity.

UTILIZATION OF ELEMENTS OF MINERAL NUTRITION BY CHLORELLA CELLS IN INTENSIVE CULTIVATION

Ye. K. Lebedeva, G. I. Meleshko and A. N. Shakhova

The use of the photosynthetic activity of green algae for the supply /687 of oxygen and some food for spacecraft crews during long flight and for the elimination from the atmosphere of the carbon dioxide produced in the process of human metabolic activity is gaining increasing importance.

In this connection it is important that a constant high level of oxygen and biological mass production by the algae be maintained in the space vehicle for a lengthy period of time, and that the chemical composition of the cells be kept unchanged. Such a constancy of photosynthetic activity can be achieved by the observance of all other optimal cultivation conditions only with a continuous maintenance of a certain concentration of mineral elements in the nutrient solution.

It is a known fact that the nutrient media used for growing various chlorella strains differ from one another by their chemical composition, salt concentration and pH. Here the growth of certain strains may continue without any marked diminution of intensity when the total salt concentration is changed within the range of 0.1-0.1 M (sic) and more (1.12), while other strains, for example, are highly sensitive even to a relatively small change (2-4 times) in a salt concentration (ref. 19). The pH itself has no effect on the rate of growth within the range of 4-9 (ref.

At the same time, a change in the composition of the culture medium produces considerable fluctuations in the chemical composition and the content of organic matter and ash elements in the cells. The shortage of nitrogen is particularly effective in a culture medium designed to produce a biological mass with a /688 high fat content (up to 80 percent) and a sharply reduced albumin content (to 20 percent and less). The total ash content of the cells, usually amounting to 7-10 percent, may increase, in some cases to 33 percent, or drop to 3.3 percent by dry weight (refs. 8, 9 and 11).

The accumulation of basic mineral nutritive elements in the cells of various chlorella strains in different compositions of the culture medium may also fluctuate within a wide range, amounting to 3.3-11.6 percent in the case of nitrogen, 0.9-2.76 percent for phosphorus and 0.1-1.6 percent for potassium (refs. 3, 7, 8, 9, 16, 17 and 22).

The maintenance of a constant composition of the culture medium in the process of algae cultivation requires periodic corrections by way of adding a certain quantity of salt, in accordance with the loss of the mineral nutritive elements.

In actual practice, the introduction of various elements into the low-productivity continuous culture media, maintained in industrial installations, is usually guided by the data on the elementary chemical composition of the biological mass. However, in the case of an intensified algae cultivation process the quantity of metabolic products released by the cells into the medium may increase, thereby producing a disparity between the observable loss of elements in the medium and their accumulation in the accretions of biological mass.

Chlorella was cultivated in a Tamiy medium prepared on distilled water and containing 5 g KNO_3, 2.5 g $MgSO_4 \cdot 7\ H_2O$ and 1.25 g KH_2PO_4 per liter, as well as iron (1.2 mg Fe^{+2}) and microelements which can be stabilized, according to Arnon's prescription (ref. 6), by the addition of EDTA, ethylenediaminetetraacetate (37 mg).

The inoculative material was cultivated beforehand in a luminostat, then centrifuged and suspended in a fresh medium.

The chlorella was cultivated in an enclosed airspace at a temperature of 39-40°C and with a 3-5 percent carbon dioxide concentration; the suspension was illuminated around the clock.

The increment of the biological mass by dry weight, the optical density of the suspension as well as the pH and concentration of the elements in the medium were determined periodically in the course of the tests. The amount of nitrogen in the form of an NO_3^- ion was established by the disulfophenolic method, phosphorus was determined by the calorimetric method and the use of phosphorotungstic acid, magnesium and sulfur were measured by a complex set of methods, potassium by the method of flame spectrophotometry and iron by the ammonium variant of the sulfosalicylic method.

In the analysis of the dry material, the organic nitrogen was determined by Kjeldahl's micromethod, and organic phosphorus and sulfur, potassium, magnesium and iron were determined by this method after a wet calcination with ammonium nitrate, as recommended by the Commission on Analytical Methods (ref. 4). The total ash content in the samples was established by calcinating them in a crucible. The cultivation of chlorella without adjusting the medium revealed a gradual change of the pH from 6.6-8.8-9.0, which did not, however, affect the rate of growth amounting to 0.5-0.6 billion cells per milliliter in 24 hours. In a number of tests the medium was adjusted by the use of nitric acid to pH 6-7, with a view to preventing the elimination of magnesium and phosphorus from the solution in the form of salts difficult to dissolve. /689

The concentration of elements in the chlorella cultivation process remained within the following range: nitrogen 0.70-0.05 g/liter, phosphorus 0.30-0.10, sulfur 0.32-0.22, potassium 1.2-1.15, magnesium 0.24-0.18 and iron 0.0012-0.0001 g/liter.

We determined the ash content in the biological mass in the course of six tests. The fluctuation of the ash content ranged from 65-80.8 mg per g of dry weight, that is, an average of 73.2 mg, which corresponds to the data found in literature (refs. 8 and 10).

The elementary chemical composition of the chlorella biological mass, as established in various tests, is presented in table 1.

According to the figures listed in table 1, the nitrogen, phosphorus, sulfur and potassium content in the biological mass is characterized by a high degree of stability; its deviation from the mean value in our series of tests did not exceed 7 percent. The magnesium content was also relatively stable, even though its fluctuation was somewhat higher and the deviation /690 from the mean value was about 20 percent. The widest discrepancy was found in the iron content, apparently due to the rapid diminution of its concentration in the medium during the tests.

Our data on the accumulation of nutritive elements in the Chlorella pyrenoidosa S-39 cells were close to those obtained by Geoghegan (ref. 7), who experimented with Chlorella vulgaris, and Scott (ref. 16), who studied the effect of the concentration of the mineral nutritive elements on their content in the Chlorella pyrenoidosa cells.

TABLE 1. ELEMENTARY CHEMICAL COMPOSITION OF CHLORELLA BIOLOGICAL MASS (IN PERCENT OF DRY WEIGHT).

Test No.	N	P	S	K	Mg	Fe
1	7.87	1.80	1.07	1.50	0.68	0.01
2	7.70	1.80	1.18	1.68	0.48	0.03
3	8.20	1.70	1.06	1.60	0.55	0.02
4	8.20	1.65	--	1.63	0.48	0.05
5	--	1.76	1.19	--	--	--
Average	8.00 ± 0.21	1.74 ± 0.05	1.12 ± 0.06	1.60 ± 0.05	0.57 ± 0.08	0.03 ± 0.01

The establishment of the chemical composition of the chlorella biological mass cultivated in a Tamiy medium facilitated an approximate calculation of the corrective additions to the medium required for the maintenance of a lasting Chlorella pyrenoidosa S-39 culture medium. We also wanted to estimate the magnitude of the error resulting from underestimating the losses of the nutritive elements by the cell by releasing them into the medium with the metabolites (refs. 6, 12-15, 18, 20, and 22).

In view of the chemical composition of the known chlorella metabolic products (amino acids, amides, chlorophyllides, unsaturated fatty acids, etc.), it was possible to expect a certain discrepancy between the elimination of the nitrogen from the medium and its accumulation in the biological mass and, to a lesser extent also, phosphorus and magnesium. In this connection, the concentration of these elements in the medium was determined periodically in the process of the chlorella cultivation, and their elimination per 100 million cells and 1 g of dry weight of the biological mass was calculated. The resulting data are listed in tables 2 and 3.

It should be pointed out that the nitrogen elimination magnitudes cited in table 2 remained constant throughout the entire experiment without regard to the changing pH.

A comparison of the magnitudes characterizing the elimination of /691
nitrogen from the medium and its accumulation in the biological mass shows that the discrepancy between them is very small, amounting to an average of 3.5 percent at a maximum deviation of 7 percent.

The reaction of the medium had to be stabilized and the pH kept from rising above 7.3 to prevent the precipitation of phosphates, in order to determine the phosphorus and magnesium. By observing that condition we succeeded in obtaining a satisfactory reproducibility of determinations in the series of tests, the results of which are shown in table 3.

A review of the figures listed in table 3 reveals a marked discrepancy between the magnitudes of magnesium elimination in the various tests, amounting to 23 percent of the mean value. The elimination of phosphorus was considerably more stable, which is also in keeping with the greater stability of its content in the dry material.

It should be admitted that an evaluation of the correlation between the average phosphorus and magnesium eliminations from the medium (table 3) and the accumulation of these elements in the biological mass (table 1) show that there is practically no difference between them. In the case of nitrogen, as pointed out earlier (table 2), the difference between the mentioned magnitudes is also small, and does not exceed 7 percent.

The continued stability of the chemical composition of the cells and the total quantity of the metabolic products during the cultivation of chlorella in certain invariable conditions facilitates a fairly precise calculation of the composition of the corrective solutions to be added to the nutrient /692
medium on the basis of the average figures. Such a method greatly simplifies

TABLE 2. ELIMINATION OF NITROGEN FROM THE TAMIY MEDIUM AND ITS ACCUMULATION IN THE CHLORELLA BIOLOGICAL MASS.

Test No.	Dry weight of 100 million cells	Nitrogen elimination from medium in mg		Nitrogen content in biological mass	
		Per 100 million cells	Per gram of dry weight	Per gram of dry weight, mg	In % of elimination
1	0.76	0.060	81.0	78.8	97
2	0.66	0.057	86.0	84.0	98
3	0.73	0.057	78.0	78.0	100
4	0.80	0.066	82.5	78.1	94.5
5	0.73	0.060	82.5	77.0	93.3
Average	0.73 ± 0.03	0.061 ± 0.004	82.0 ± 1.9	79.1 ± 1.9	96.5 ± 2.1

TABLE 3. MAGNESIUM AND PHOSPHORUS ELIMINATION FROM THE TAMIY CULTURE MEDIUM.

Test No.	Magnesium	Phosphorus	Magnesium	Phosphorus
	mg per 100 million cells		mg per gram of dry weight	
1	0.0039	0.0140	4.80	17.80
2	0.0054	0.0156	7.35	21.00
3	0.0048	0.0104	6.55	14.20
4	0.0050	0.0121	6.95	16.50
5	0.0030	0.0094	4.05	12.80
6	0.0050	0.0126	6.40	17.30
7	0.0030	0.0149	6.05	18.40
8	0.0050	--	6.80	--
9	--	0.0150	--	17.90
Average	0.0044 ± ± 0.0008	0.013 ± 0.0016	5.75 ± 1.20	17.60 ± 1.70

the maintenance of a stable composition of the cultural medium, obviating the necessity of a continuous instrument analysis of all the elements and a programmed control of the composition of the corrective solution.

Shown in table 4 are the absolute quantities of the nutritive macroelements, which should be contained in the solution designed to correct the medium during the Chlorella pyrenoidosa S-39 cultivation, as well as the molar and weight relations between these elements.

TABLE 4. QUANTITY OF MINERAL NUTRITIVE ELEMENTS ADDED TO THE NUTRIENT MEDIUM PER GRAM OF BIOLOGICAL MASS INCREMENT.

Element	Quantity of element		Relative magnitude	
	mg/g	(mg atom/g)	Weight ratio	Molar ratio
Nitrogen	82.0	5.85	1	1
Phosphorus	17.4	0.56	0.212	0.095
Sulfur	11.2	0.35	0.136	0.060
Potassium	16.0	0.42	0.195	0.072
Magnesium	5.7	0.24	0.070	0.041
Iron	0.3	0.0056	0.0037	0.001

As indicated in table 4, the chlorella growth requires a fairly large quantity of nitrogen, considerably larger than its phosphorus, sulfur, magnesium and potassium requirements.

Thus the weight ratio between the nitrogen and phosphorus consumption is 4.1 : 11, and the molar ratio 11:1. The molar ratios between the nitrogen, phosphorus, potassium, sulfur and magnesium quantities (N:P:K:S:Mg) are, respectively, 25:2, 3:1, 75:1, 5:1.

Table 4 also provides an idea of the extent of the ion imbalance occurring in the process of chlorella cultivation as a result of the difference in its anion and cation consumption. Thus, the total NO_3^-, SO_4^{-2} and $H_2PO_4^-$ anions consumed by the chlorella amounts to 7.11 mg·ekW, and the total cations (K^+ and Mg^{+2}) was only 0.9 mg·ekW per 1 g of biological mass increment, which accounts for the higher pH. The culture medium should, therefore, be corrected by the use of mixtures consisting of neutral salts and free acids. Listed in table 5 are the absolute quantities of the compounds, per gram of biological mass increment by dry weight, which are added to the nitrate medium with a view to correcting its element concentration and the pH.

The figures listed in table 5 can be used as a basis for calculating /693 the composition of the corrective solutions for different cases of lengthy and intensive cultures of Chlorella pyrenoidosa S-39 in nitrate media involving the preservation of the optimal concentrations of the nutritive mineral macroelements.

TABLE 5. ABSOLUTE OXYGEN AND SALT QUANTITIES ADDED TO NUTRIENT MEDIUM PER GRAM OF BIOLOGICAL MASS INCREMENT.

Compound	nM	g	Compound	nM	g
HNO_3	5.85	0.300	$MgSO_2 \cdot 7H_2O$	0.24	0.059
KH_2PO_4	0.42	0.057	H_2SO_4	0.11	0.010
HP_3O_4	0.12	0.011	$FeSO_4 \cdot 7H_2O$	0.0056	0.0015

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CHANGES IN HEMATOCRIT INDICES AND GAS COMPOSITION OF ARTERIAL BLOOD IN WHITE RATS DURING ARTIFICIAL HYPOTHERMIA

G. D. Glod

The organism is supplied with oxygen by a complex interaction of /694
numerous systems, with the respiratory and blood-circulation organs playing a major role. The development of a state of artificial hypothermia is accompanied by a growing inhibition of the basic physiological functions, along with a reduced energy expenditure. The normal vital activity of a highly organized animal is possible only with homeostatic correspondence between the functional states of the respiratory and blood-circulation organs and the energy requirements of the tissues. An investigation of the gas composition of the arterial blood makes some evaluation of this correlation possible.

The respiratory blood function of white rats with a low body temperature was studied by A. L. Izbinskiy (ref. 1), but in his investigations the supercooling method of hypothermia was used. No exhaustive investigations in this field, involving artificial hypothermia of rats with a blocked nervous system, are found in the available literature.

An attempt was made in this investigation to evaluate the effectiveness of certain methods of artificial hypothermia in white rats in connection with their respiration and the gas composition of the arterial blood under various degrees of hypothermia.

The experiments involved precise tests on 60 white laboratory rats of both sexes weighing 120-150 g. Artificial hypothermia was achieved by placing the animals in a cooling chamber with a temperature ranging from 0 to -5°C. The reduction or elimination of the reflex reactions to the cooling process in the first series of tests (23 rats) was achieved by cooling the animals in a closed and limited space under conditions of increasing hypercapnia, or hypoxia (ref. 4); in the second series (involving 10 rats) it was achieved by /695
the hypothermic method in an artificial gas medium consisting of 15 percent CO_2, 40 percent O_2 and 45 percent nitrogen (the mixture had been prepared by the anesthetic apparatus of the Krasnogvardeyets plant). The composition of the mixture was controlled by a complex Godart gas analyzer; in the third series of tests artificial hypothermia was achieved by blocking the afferent signal system before the cooling process by the intramuscular introduction of a "lytic" mixture (aminazie 7.0, "pipoflen" 5.0 and promedol 1.0 mg/kg, and a 0.75 percent solution of 7.5-10.0 mg/kg diplacin to prevent tremors).

Arterial blood specimens of the control animals (4 rats) were taken from a puncture in the carotid artery in doses of 1.5-2.0 milliliters once in every stage of hypothermia under normal body temperature, with the animals in a state of slight ether anesthesia. The temperature was also taken rectally at the same time (with a thermometer working on the thermocouple principle), and the frequency of the respiratory movements of the thorax was recorded.

The blood specimen under the oil was mixed with a dry anticoagulant (potassium citrate). The hematocrit reading was checked (by centrifuging the device for 15 min at 3,000 rpm), and the gas composition investigated by Van Slyke's method (oxygen and carbon dioxide content). Blood specimens for hematocrit testing were taken from the tails of some of the animals (ref. 27) before the hypothermic process.

The results of the investigations were grouped according to body temperature and respiration frequency, and subjected to statistical processing by the small sample method (ref. 2). Calculations were made of the mean values in groups (M), their average errors (± M), the authenticity indicators of the mean values (P) and the authenticity indicators of the differences between the comparable mean values (t).

The characteristic features of the development of artificial hypothermia in the test animals were determined largely by the method of blocking the nervous system. The differences were most pronounced in the initial period of the hypothermia, up to a temperature of 30-28°C. A brief stage of motor stimulation, which soon gave way to a depression on the part of the animal, was observed in the first series of tests. In the second series the stimulation was of longer duration, and the respiration rate became considerably higher with the onset of hypothermia. There was no stage of stimulation in the third series of tests. Following the introduction of the "lytic" mixture, the rats calmed down, grew sluggish and lost some mobility; the introduction of diplacin produced a pronounced muscular relaxation. Kept in the same environmental conditions (-5°C), the rats were cooled to a temperature of 20-18°C in 1.5-2 hours in the first series and in the third series in 40-50 min.

The appearance of the animals whose temperature had been reduced below 28-25°C was about the same by all methods of artificial hypothermia under study. With the exception of occasional tremors in their extremities, the rats were fully relaxed and did not change the lateral position in which they had been placed. There was a rose-colored tint on the skin of their paws. The /696
algesia diminished to a considerable extent, but the tactile sensitivity remained. Respiration rhythm was disturbed when the body temperature decreased to 18-16°C, and in their third series of tests it ceased in most of the animals. By that time the frequency of cardiac contractions did not exceed 60-80 per min. A continued hypothermic process (to 16-14°C) led to considerable disturbances and stoppage of respiration also in the first two series of tests. The considerable individual differences in the animals' sensitivity to artificial hypothermia should be pointed out.

The following changes in the blood concentration produced by the artificial hypothermia methods under study were revealed by the blood tests (fig. 1). The changes indicated by the hematocrit in the first series of tests were of a two-phase nature. The drop in body temperature of the test animals was accompanied by a condensation of the blood; the hematocrit index rose up to ($t = 2.7$) from 45.1 ± 1.09 ($P < 0.001$) in the initial state to 50.5 ± 1.66 ($P < 0.0001$) after a temperature drop to 23-19°C. Further hypothermia was accompanied by moderate rarefaction of the blood. At a temperature of 19-15°C, for example, the hematocrit index decreased ($t = 3.1$) to 40.3 ± 2.8 ($P < 0.001$), and below 15°C to 36.4 ± 2.55 ($P < 0.001$). The changes in the blood concentration of the animals in the second series of tests, as their temperature decreased to 27-23°C and 23-19°C were about the same as in the first series; a deeper hypothermia revealed a further concentration of the blood. The changes in the hematocrit index were somewhat different in the third series of tests. The drop in body temperature was accompanied by a gradual rarefaction of the blood from the very /697
beginning; at 35-31°C the hematocrit indicator decreased to 42.3 ± 3.29 ($P < 0.01$), at 27-23°C to 41.7 ± 3.64 ($P < 0.01$), at 23-19°C to 41.0 ± 2.82 ($P < 0.001$), and at 19-15°C to 40.0 ± 1.81 ($P < 0.001$). The difference between the mean value in the initial condition and the mean value under maximum hypothermia is substantial ($t = 2.4$).

The changes in the gas composition of the arterial blood under the three methods of producing artificial hypothermia amounted to the development of artificial hypoxemia. Its prominence varied with the different methods (fig. 2). The drop in body temperature to 25-20°C in the first series of tests resulted in a reduction ($t = 1.8$) in the quantity of oxygen from 19.77 ± 0.9 ($P < 0.001$), and at normal body temperature the reduction was to 13.21 ± 3.46 vol. percent ($P < 0.05$). Further hypothermia (to 20-15°C) revealed little change in O_2

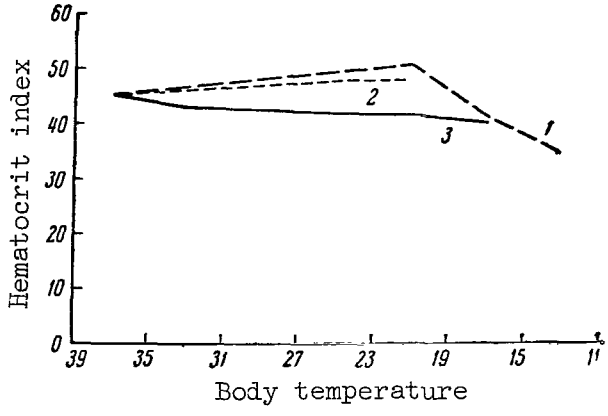

Figure 1. Change in hematocrit index in white rats produced by artificial hypothermia: 1, results of first series of tests; 2, second series; 3, third series.

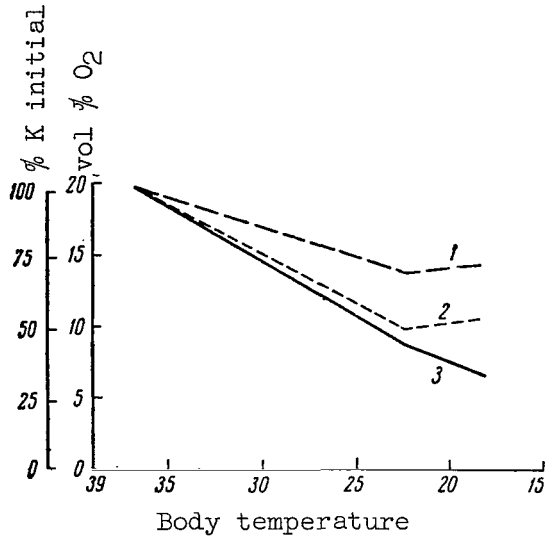

Figure 2. Change in oxygen content in arterial blood of white rats (in volume percent) produced by artificial hypothermia. Symbols are the same as in figure 1.

(14.31 ± 2.7 vol. percent; $P < 0.01$). In the second series of tests the changes were more pronounced; at 25-20°C the amount of oxygen in the arterial blood was reduced ($t = 4.24$) on the average of 9.71 ± 2.37 volume percent; ($P < 0.05$), and at 20-15°C the quantity of O_2 remained about the same (10.55 ± 2.44 vol. percent; $P < 0.05$). The greatest arterial hypoxemia was noted in the third series of tests. A drop in temperature to 20-15°C resulted in a reduction ($t = 6.05$) of O_2 in the arterial blood to 6.5 ± 1.97 vol. percent ($P < 0.05$).

In our experiments the amount of oxygen in the arterial blood was apparently determined to a considerable extent by the gaseous metabolism in the lungs, as indicated by comparison of the results with the changing frequency of the respiratory movements (fig. 3). /698 It may be seen from figure 3 that at a respiration frequency of 15 per minute, the amount of oxygen in the arterial blood in the first two series of tests was practically unchanged, and in the third series it was reduced by about 25-30 percent. Further inhibition of the respiration revealed more pronounced difficulties, as the amount of O_2 in the arterial blood in the first series of tests, when the respiration frequency was 15-6 per minute, remained at approximately the previous level, while in the third series of tests it dropped to 45-50 percent of the initial level (the number of observations and the variability of the groups were approximately

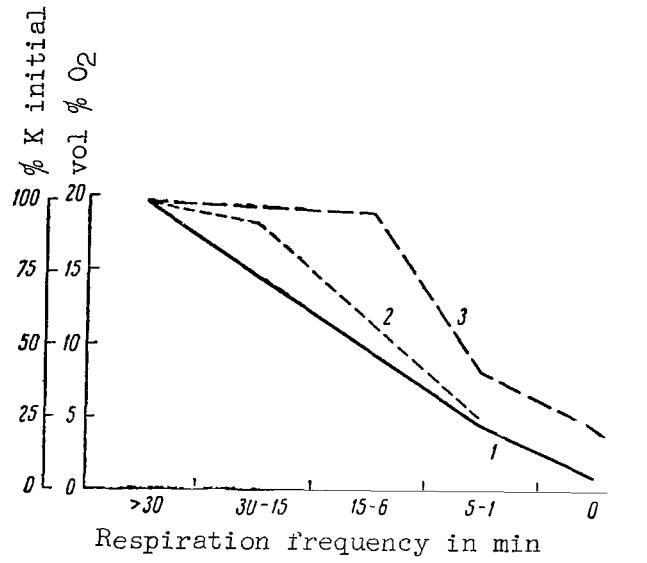

Figure 3. Comparative data on the changing oxygen content in the arterial blood of white rats (in volume percent) determined by the frequency of respiratory movements. Symbols are the same as in figure 1.

the same). The amount of oxygen was sharply reduced in all three series of tests by further inhibition of the respiration (a frequency of less than 6 per minute).

The development of arterial hypoxemia was accompanied by a changing quantity of carbon dioxide in the blood. No "accumulation" of carbon dioxide in the blood was observed in the first series of tests, while a minor hypoxemia even revealed a reduction ($t = 2.4$) from 40.1 ± 0.9 ($P < 0.001$) in the initial state to 32.7 ± 2.92 vol. percent ($P < 0.001$). In deeper hypoxemia the quantity of CO_2 in the blood approached the initial level. There were no changes in the amount of CO_2 in the blood in the second series of tests (when the amount of oxygen was reduced to about one-half). A deeper hypoxemia increased the amount of carbon dioxide ($t = 2$) to 61.2 ± 7.81 vol. percent ($P < 0.01$). The amount of CO_2 in the arterial blood of the rats in the third series of /699 tests was found to increase gradually with developing hypoxemia. With the oxygen content in the blood above 7 vol. percent, it increased ($t = 2.03$) to 49.0 ± 4.28 ($P < 0.001$), and when the O_2 content was less than 7 vol. percent, it increased ($t = 3.42$) to 54.2 ± 4.02 vol. percent ($P < 0.001$).

Thus the results of our investigation showed that artificial hypothermia in white rats can be achieved by any of the methods under study, but that the characteristic features of hypothermia are determined by the methods of producing it. For example, the duration of hypothermia was shortest in the tests involving the use of a "lytic" mixture and diplacin, and longest in hypercapnia conditions. This is indicative of the complete blockage of afferent signals in the tests of the third series. In the development of artificial hypothermia by this method, however, the inhibition of the basic vital functions, with the hypothermic intensity being equal, was more pronounced than in the tests of the first two series, as indicated by the early inhibition of the respiratory function.

The oxygen supply conditions in cases of artificial states of hypothermia differing little in appearance and similar in intensity were not similar. All methods of artificial hypothermia resulted in development of arterial hypoxemia, most pronounced in the third series of tests (30-35 percent of the O_2 level at a body temperature of 20-15°C). The second series of tests occupied an intermediate position. Finally, in the first series the amount of O_2 in a similar state of hypothermia was the highest (70-75 percent of the initial level). It appears that the disruption of external respiration was one of the major causes of arterial hypoxemia observable in our experiments.

A review of the gas composition of the blood in its relation to the frequency of respiratory movements shows that the external respiration by the methods of artificial hypothermia under study is qualitatively different. The conditions of the blood oxygenation in the lungs were more optimal in the first two series of tests, even when a considerable decrease in breathing frequency was not accompanied by pronounced hypoxemia. It is obvious that the frequency of respiratory movements of the rats in our experiments did not adequately reflect pulmonary ventilation. This justifies the assumption that the disruption of external respiration in the third series of tests occurred when the frequency of respiratory movements was still fairly high.

The assumption is supported by the examination of the CO_2 content in the arterial blood. The capacity of the organism to rid itself of one of the chief products of tissular metabolism was disrupted to a very large extent in the series of tests, which was apparently also indicative of considerable disruption of the gaseous metabolism in the lungs, and possibly also disruption of the ferment systems which facilitate the transfer of the carbon dioxide /700 by the blood and its release. The organism's capacity to eliminate carbon dioxide was not affected in the first series of tests. The compensating mechanisms were observed to be mobilized early in the development of hypoxemia, and the result was an insufficiently large release of carbon dioxide. That was apparently due to the specific effect on the animal's organism in the tests of that series by the gradually increasing hypercapnia, or hypoxia, and the mobilization of the physiological systems responsible for the release of carbon dioxide from the organism. A similar picture was also observed in the second series of tests, but only in the initial stage of developing hypothermia, when

the carbon dioxide content in the arterial blood was also somewhat lower. Deeper hypothermia resulted in sharp increase of the CO_2 content in the blood.

The hypoxia-producing gas mixture in these tests probably brought about mobilization of the compensating mechanisms only to a certain level of hypothermia, whereupon its second stage set in and the compensating mechanisms were activated.

In our experiments the hematocrit index changed in all three series of tests; a distinct tendency to blood condensation was noted in the first two series, and to hydremia in the third series. Without referring to the possible mechanisms of these changes, we merely point out that they could not substantially improve the transfer of gas by the blood, inasmuch as both the relative quantity of erythrocytes and the "regular elements-plasma" correlation did not change much.

The results of our investigation justify the consideration of these methods of artificial hypothermia in white rats from a comparative aspect, whereby the method of hypothermia under conditions of growing hypoxia was found to be most effective. The results of our investigation support the studies by a number of authors showing the effective use of increasing hypoxia in connection with artificial hypothermia of white rats (refs. 4-6).

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MODEL OF RADIATION CONDITIONS ON A CIRCUMLUNAR TRAJECTORY DURING A SOLAR FLARE

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P. P. Saksonov and N. N. Dobrov

A further development of rocketry will make manned flights to the /701
other planets of the solar system possible in the near future. But before this happens, a detailed biological study should be made of the proposed flight routes, in addition to a study of the physical characteristics of cosmic radiation, with a view to reducing the flight-connected risks to a minimum. One way of exploring the future flight trajectory will be the launching of space vehicles carrying animals and other biological objects aboard. The preparation for such a flight, in turn, will call for a large number of experiments under terrestrial conditions. The establishment of the most probable biological effect produced by relatively lengthy spaceflights under the most unfavorable radiation conditions calls for a definite program of modeling the effect of ionizing radiation as well as certain other factors (vibration, acceleration, confined space and changing gaseous composition in the cabin) which can now be carried out under laboratory conditions.

The experiments involving the model of a spacecraft flight around the Moon, with a possible approximation, should be carried out as the first stage. These should take into account the most unfavorable radiation conditions and the occurrence of solar flares.

It is known that solar flares producing high fluxes of charged particles, mostly protons with energies ranging from 10 million to several billion electron volts, occur periodically on the solar surface. These conspicuous phe- /702
nomena of solar physics take place at certain periods of time and are statistically associated with the active regions on the Sun. The charge particles of solar origin spread into space at various speeds under the effect of interplanetary magnetic fields.

In the area of Earth these particles appear in substantial quantities (10-20 percent of the flare time); then their intensity drops to the usual cosmic radiation background. The time it takes the intensity to reach a maximum point varies from 0.5-1.5 hour. The intensity drops to the usual cosmic radiation level in the next 10-20 hours. The total particle flux per flare may consist of 10^6 to 10^9 particles per cm^{-2}, and the duration of the solar flares varies from several hours to several days. Present in the particle flux, in addition to protons, are α-particles and the nuclei of the C, N and O group

elements (ref. 4). The ratio of number of protons to the α-particles may be above 40, and to the number of C, N, O nuclei from 10^2 to 10^3.

The completed investigations (ref. 1) justify the assertion that about 3 percent of all large flares are potentially dangerous to spaceflights. The number of such flares in times of minimum solar activity is about 3 a year, and maximum activity about 100 a year.

Thus the radiation danger to spaceflight from solar flares is fairly serious, and it quite obviously increases with the increasing duration of the flight, which is accompanied by a cumulative dose of cosmic radiation.

The tentative data on the possible doses produced by three types of flares through different layers of water (in $g \cdot cm^{-2}$) are presented in table 1 (refs. 2 and 3).

It may thus be assumed that if the protective layers of a space vehicle are less than 4-5 $g \cdot cm^{-2}$, the crew would be exposed to a radiation dose above 100 rad in case of dangerous flares. This emphasizes the problem of developing reliable protection for the space vehicle against ionizing radiation. /703

The flight of a space vehicle on a trajectory around the Moon can be divided into several stages. The first stage would be overcoming the radiation belts in the course of 2.5 hours (ref. 2), during which time the crew would get a dose of about 5 rad (ref. 5). The second stage would be the flight beyond the radiation belts, during which the major and lasting effect would be produced by cosmic radiation of 8-12 m per day (ref. 6). The third stage would be modeled as the beginning of long-range prognosis, which now amounts to from one to three days (ref. 7). The possible radiation dose to which the crew might be exposed would depend on the duration of the flare, its class, the intensity of the charged particle flux and its energy, as well as the type of the spacecraft protection. The magnitude of the dose may vary within a very wide range, from several dozen to several thousand radians. Certain initial data related to the problems under study should therefore be selected for the modeling experiments. The fourth stage, the return trip, presents a very serious danger as the vehicle goes into orbit around Earth before landing, spending some time in the radiation belts. The dose in such a case may amount to several dozen radians.

The problem of spaceflights to other planets of the solar system lasting over a year is a matter of great interest from the point of view of cosmic radiation and the development of an effective protection against it. In the course of a lengthy flight, the crew will be constantly under the effect of primary cosmic radiation and dangerous periodic solar flares. The modeling of short flights should therefore be accompanied by lengthy experiments on the ground, with a view to studying the effect of ionizing radiation in combination with several other factors on biological objects, under conditions as close to reality as possible. In this connection, it might be well to look into the possibility of modeling the biological effect of cosmic radiation in space by the

TABLE 1. RELATIONSHIP BETWEEN THE DOSE AND THE PROTECTIVE LAYER (g/cm^2) IN THE THREE TYPES OF FLARES.

Dose, billion eV	Type of flare		
	Aug. 22, 1958	May 10, 1959	Feb. 23, 1956
1	8	--	--
5	4.2	--	--
10	3.5	34	--
15	3.3	28	--
20	3.0	27	36
25	2.8	25	32
50	2.5	19	21
100	2.0	15	13

use of terrestrial sources. What actually should be modeled is the effect of primary cosmic radiation, the solar flare protons and the emission of the terrestrial radiation belts.

The biological effect of any type of ionizing radiation is determined by comparing it with X-ray and gamma radiation, which is taken as unit 1. The effect of other types of ionizing radiation is determined by the coefficient of the relative biological effectiveness (RBE). As shown in a number of studies, the RBE of protons with an energy above 100 million eV differs little from 1.

No basic difference has been observed in the nature of the biological /704 reaction produced by the protons, as compared to X-ray or gamma radiation (refs. 8 and 9). Consequently, radioactive Co^{60} can be used, in the first approximation, as a source of ionizing radiation. There is no doubt that further experiments will require the use of a flux of protons of varying energy for radiation purposes. This will make it possible to experiment under conditions approximating reality. The model of a flux of charge particles of cosmic radiation in the laboratory, with a view to studying its biological effect on large objects, will hardly be possible in the near future. But a study of the effect of corpuscular radiation on biological objects and a definition of the RBE coefficient, especially in lengthy flights, will be most important.

A special biological unit for small laboratory animals was designed to make a preliminary experiment to model the effect of ionizing radiation under terrestrial conditions, and to meet some additional requirements connected with the maintenance of the animals in spaceflight.

The behavior of the animals was observed through special sight holes in the unit by a television device.

Appropriate model experiments were made to evaluate the biological effect of radiation produced by solar flares during spaceflight (ref. 10). A short solar flare was modeled, as well as one of the possible variant radiation conditions in a flight to the Moon. Figure 1 shows the changing duration of the dose in the "solar flare" scheme, and figure 2 shows the changing duration of the dose in the "flight to the Moon" scheme.

The tests were made with 278 nonpedigreed white mice.

A gamma-cobalt installation was used as a source of ionizing radiation. The irradiation under the "solar flare" and "flight to the Moon" schemes took place in a special biological unit. The animals used in this case were fed a special food concentrate. The acute exposure took place in organic glass cages. The integral radiation dose in all cases amounted to 900-920 r. In the acute exposure, the dose was 18 r per minute, and in the exposure under the "solar flare" and "flight to the Moon" schemes the dose was varied according to a preset program (figs. 1 and 2). /705

The maximum dose rate in a flare amounted to about 2.5 r per minute. The duration of the flare was 24 hours and the total dose 800 r. The integral dose

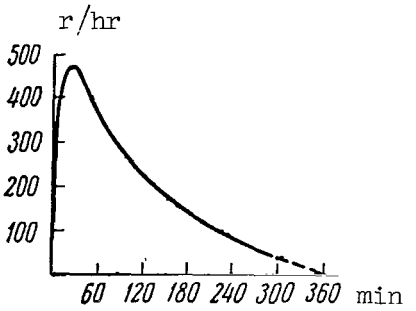

Figure 1. Changing dose rate during modeling of "solar flare."

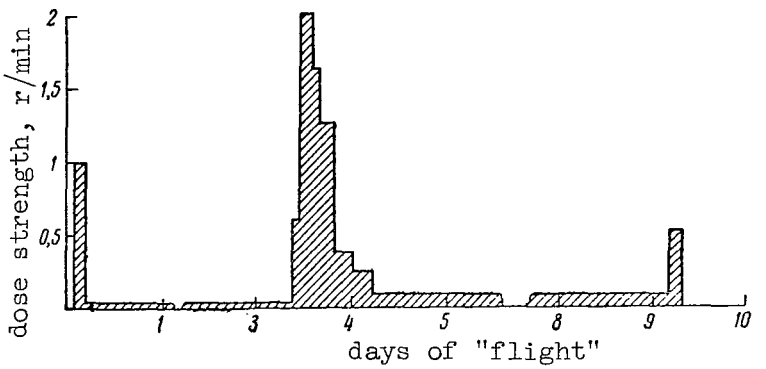

Figure 2. Changing duration of dose rate under "flight to Moon" scheme.

during the flight through the "radiation belts" in one direction was, according to the scheme adopted by us, 60-80 r.

Under the "flight to the Moon" scheme, the animals were subjected to an acceleration effect of 20 g for 5 min before the irradiation and at the end of it. The following radioprotective preparations were introduced into the animals intra-abdominally before the "solar flare," under the "flight to the Moon" scheme: cystamin dihydrochloride (150 mg/kg), 3,β-aminoethylisothiuronium bromide dihydrobromide (AET, 150 mg/kg), 5-methoxytryptamin (mexamin) hydrochloride (75 mg/kg). The preparation doses were taken as a base for calculations.

The resulting experimental data are presented in tables 2 and 3.

As indicated in table 2, there was little biological difference between the radiation modeling under the short "solar flare" scheme and the acute exposure to a similar total dose.

The effect of the dose rate was clearly pronounced in radiation in /706
the "flight to the Moon" scheme. If the death rate produced by the acute exposure was 90 percent, the irradiation under the "flight to the Moon" scheme was followed by a death rate of only 33 percent. In that scheme the irradiation represents a combination of fractional and extended irradiation.

It is our opinion that the reduction in the biological effect of ionizing radiation under the "flight to the Moon" scheme is determined not only by the dose rate, but also by the effect of the acceleration which may change the animal's reaction to subsequent ionizing radiation (ref. 11). It may be interesting to point out that a recently published study reveals that preliminary irradiation with a dose of 50 r increases the DL_{50} in mice from 487 (within the norm) to 560-617 r (ref. 12). It may be assumed that in our experiments the preliminary irradiation with a 60 r dose has somewhat reduced the biological effectiveness of subsequent irradiation with a higher dose.

The use of pharmacological remedies (AET, cystamin, mexamin) in the radiation under the "flight to the Moon" scheme provided some protection, but it was not very pronounced in comparison with radiation without protection. It is interesting to point out that the percentages of deaths among the animals protected by pharmaceuticals and chemical preparations against acute exposure and irradiation under the "flight to the Moon" scheme were of the same order: 10 and 7 (AET), 25 and 14 (cystamin), and 35 and 20 (mexamin), respectively.

The most pronounced radioprotective effect was produced by the use of AET and, to a lesser extent, mexamin. These correlations also remained unchanged in the "flight to the Moon" scheme.

The experimental investigations under discussion were not designed to /707
provide an exhaustive explanantion for every possible type of radiation in spaceflight. Such experiments will be the object of our further investigations.

TABLE 2. SURVIVABILITY AND LIFESPAN OF MICE IRRADIATED UNDER THE "SOLAR FLARE" SCHEME.

Test conditions	No. of animals	Died by 30th day	Percent of deaths	Average lifespan
"Solar flare" dose 900 r (concentrated food)	20	15	75	12.6 ± 0.8
Acute exposure to 900 r (concentrated food)	20	15	75	10.8 ± 1.2
Acute exposure to 900 r (usual vivarium-type food)	20	18	90	11.5 ± 1.1
Biological control	20	-	-	-

TABLE 3. PROTECTIVE EFFECT OF CHEMICALS AGAINST THE IRRADIATION UNDER THE "FLIGHT TO THE MOON" SCHEME.

Test conditions	No. of animals	No. of mice dead by 40th day after radiation, in %	Average lifespan of mice that died, M ± m*
Irradiation under the "flight to the Moon" scheme			
920 r	18	33	11.2 ± 0.6
920 r + AET	15**	7	8.0
920 r + crystamin	15**	14	15.0 ± 5.0
920 r + mexamin	20	20	12.8 ± 2.4
Single exposure			
920 r	20	90	9.8 ± 0.5
920 r + AET	20	10	18.0 ± 7.8
920 r + cystamin	20	25	11.8 ± 2.0
920 r + mexamin	20	35	12.7 ± 0.9
Biological control No. 1	20	-	-
Biological control No. 2	20	-	-

*Mean standard error.
**Mice died after introduction of preparation.

Remark: The animals included in control No. 1 were kept in a vivarium, fed concentrates and, as in the case of irradiation under the "flight to the Moon" scheme, were centrifuged twice with a 10-day interval between operations. Control No. 2 was the usual biological control.

The purpose of this study was to show that it is possible in principle to evaluate the biological effect on the Earth-Moon trajectory under modeling conditions. It should be pointed out that it is theoretically possible to model any spaceflight trajectory not only by varying the duration of the radiation dose, but also by changing the type of particles and energy spectrum of the solar flares. We believe that an analysis and evaluation of such modeling possibilities are both important and necessary.

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CONCLUSIONS

Less than a year has passed since the publication of the third volume of the collection "Problemy kosmicheskoy biologii" (Problems of Space Biology) /709
1964. During this time tremendous progress has been made in the study and conquest of space. The first multimanned orbital flight was carried out on the new Voskhod spacecraft. The participation in this historic flight of the physician-experimenter B. Yegorov, who executed an extensive program of physiological investigations during the flight, was of exceptional significance for space biology and medicine.

The flight of P. Belyayev and A. Leonov on Voskhod 2 was of unusual scientific and practical importance, for it marked the first time that man walked in free outer space. The results of this flight clearly demonstrated the present high level of technology and the maturity of aviation and space medicine. During all phases of the flight and while the walk in space was taking place the cosmonauts brilliantly carried out the complex flight plan in full possession of their faculties. The investigations in the course of the flight of Voskhod 2 were a major contribution to the solution of several theoretical problems in space science (orientation and biomechanics in unsupported space, "physical fitness" in weightlessness, etc.) and a source of encouragement for further research in this field.

The Americans who flew in Gemini 3 and Gemini 4 also made a great contribution to manned spaceflight. During the flight of Gemini 4 Edward White walked in space, testing a jet device as an aid in locomotion.

Accurate and complete information on the physics of spaceflight is enormously important for the successful development of space biology. Consequently, the latest investigations on the satellites Kosmos, Electron, Polet 2, Ranger, etc., are worth noting. Zond 2 is continuing its journey to the planets /710
of the solar system. Mariner 4 transmitted pictures of Mars to Earth.

The past year has been marked by the increasing participation of other countries besides the Soviet Union and United States in space research. Their scientists have been greatly helped by scientific contacts and growing exchange of information at international congresses and symposia.

Soviet space biologists have actively participated in the meetings of COSPAR (Florence, 196 and Buenos Aires, 1965), Congress of the International Astronautics Federation (Warsaw, 1964), Congress of Aviation and Space Medicine (Dublin, 1964), International Symposium on Bioastronautics (San Antonio, 1964), Symposium on Radiobiology (Brno, Czechoslovakia, 1965), and the Eleventh Meeting of the American Astronautics Society (Chicago, 1965). Considerable

attention was paid to aspects of space physiology at the All-Union Congress of Physiologists (Yerevan, 1964) and at some other conferences. The first Symposium on the Use of Mathematical Methods in Aviation and Space Medicine (Moscow, 1965), organized by the Council on Cybernetics, USSR Academy of Sciences, is also worth recalling.

The interest and friendly welcome accorded the previous numbers of Problemy kosmicheskoy biologii (Problems of Space Biology) has led the Editors to hope that this collection will also be useful in broadening the research front and in encouraging more and more scientists to work on the fascinating and vital problems of space biology.

The Editors express deep regret on the untimely death of their colleague Professor Andrey Vladimirovich Lebedinskiy, member of the USSR Academy of Medical Sciences. Professor Lebedinskiy was an outstanding authority in the field of aerospace medicine, one of the pioneers in this field in the Soviet Union and one of the active Editors of this collection who did much to help it come into being and ensure its success.

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